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Arginine-based poly(ester amide) nanoparticle platform: From structure–property relationship to nucleic acid delivery

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ABSTRACT

Many different types of polycations have been vigorously studied for nucleic acid delivery, but a systematical investigation of the structure–property relationships of polycations for nucleic acid delivery is still lacking. In this study, a new library of biodegradable and biocompatible arginine-based poly(ester amide) (Arg-PEA) biomaterials was designed and synthesized with a tunable structure for such a comprehensive structure–property research. Nanoparticle (NP) complexes were formed through the electrostatic interactions between the polycationic Arg-PEAs and anionic nucleic acids. The following structure effects of the Arg-PEAs on the transfection efficiency of nucleic acids were investigated: 1) the linker/spacer length (length effect and odd–even effect); 2) salt type of arginine; 3) the side chain; 4) chain stiffness; 5) molecular weight (MW). The data obtained revealed that a slight change in the Arg-PEA structure could finely tune its physicochemical property such as hydrophobicity, and this could subsequently affect the nanoparticle size and zeta potential, which, in turn, regulate the transfection efficiency and silencing outcomes. A further study of the Arg-PEA/CpG oligodeoxynucleotide NP complexes indicated that the polymer structure could precisily regulate the immune response of CpG, thus providing a new potential nano-immunotherapy strategy. The *in vitro* data have further confirmed that the Arg-PEA NPs showed a satisfactory delivery performance for a variety of nucleic acids. Therefore, the data from the current study provide comprehensive information about the Arg-PEA structure–transfection property relationship; the tunable property of the library of Arg-PEA biomaterials can be one of the promising candidates for nucleic acid delivery and other biomedical applications.

Statement of Significance

Polycations have being intensive utilized for nucleic acid delivery. However, there has not been elucidated about the relationship between polycation's structure and the physicochemical properties/biological function. In this timely report, an arginine based poly(ester amide) (Arg-PEA) library was prepared with finely tunable structure to systematically investigate the structure–property relationships of polycations for nucleic acid delivery. The results revealed that slight change of Arg-PEA structure could finely tune the physicochemical property (such as hydrophobicity), which subsequently affect the size and zeta potential of Arg-PEA/nucleic acid nanoparticles(NPs), and finally regulate the resulting transfection or silencing outcomes. Further study of Arg-PEA/CpG NPs indicated that the polymer structure could precisely regulate immuno response of CpG, providing new potential nano-immunotherapy strategy. *In vitro* evaluations confirmed that the NPs showed satisfied delivery performance for a variety types of nucleic acids. Therefore, these studies provide comprehensive information of Arg-PEA structure–property relationship, and the tunable properties of Arg-PEAs make them promising candidates for nucleic acid delivery and other biomedical applications. Overall, we have shown enough significance and novelty in terms of nucleic acid delivery, biomaterials, pharmaceutical science and nanomedicine.

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1. Introduction

Gene therapy is a method wherein foreign genes can be introduced into target cells for the treatment of genetic abnormalities or defects caused by gene defects [1–3]. As one of the most promising treatments for many important diseases, nucleic acid drugs have attracted the attention of many researchers. However, they are easily degraded by nuclease *in vivo*, thus resulting in poor biological stability, low bioavailability, and low targeting efficiency [4]. The rapid development of drug delivery technology may solve problems of the stability of nucleic acid drugs and targeted delivery to some extent. The use of vector technology and drug delivery system can improve the biological activity of nucleic acid drugs *in vivo* and enhance cellular uptake capacity [5–7].

Polycations have been intensively utilized for nucleic acid drug delivery owing to their charge condensing capability [8–11]. The underneath detailed mechanisms, however, have not been fully elucidated yet. Numerous reports have concluded that the chemical structure of the polycation carriers is the key factor that decides the nucleic acid encapsulation and delivery efficiency [3,12,13]. Limited systematical investigations have been conducted to quantitatively link the chemical structure of polycations to their physicochemical properties and their nucleic acid delivery performance [14–20]. For example, hydrophobicity of the polycation carriers has been considered as one of the fundamental properties of polycations affecting their delivery efficiency of nucleic acids. However, there have been limited reports on the quantitative evaluation of hydrophobicity and investigation of structure–function relationship, particularly the structure–biological property of the formed NP complex of polycation/nucleic acid [16,17,20]. This is mainly due to the complicated NP properties and cellular delivery routes of nucleic acids [4,21,22]. Another major factor behind the very limited systematic study of such structure–biological property relationship is the lack of suitable polycation family systems for this kind of investigation [12]. Therefore, polycation library with precisely controlled structures is highly desirable for a better understanding of the relationship between polycation structures and their biological properties for nucleic acid delivery and corresponding polycation/nucleic acid NPs. The recent availability of a new arginine (Arg)-based biodegradable and biocompatible poly (ester amide) biomaterial (Arg-PEA) provides an ideal opportunity to design a series of Arg-PEA for such a systematic and comprehensive investigation of their structure–biological property relationship for nucleic acid delivery [23].

An Arg-PEA library with finely tunable chemical structures was designed and developed. As a recently developed new class of biocompatible and biodegradable polycations, some of Arg-PEAs have been evaluated as potential nucleic acid and protein delivery carriers [24,25]. Arg-PEAs were synthesized by a condensation reaction between di-*p*-toluenesulfonic acid salts of active di-p-nitrophenyl esters of dicarboxylic acids (as bis-electrophiles) and bis-(L-α-amino acid) α,ω-alkylene diesters (as bis-nucleophiles) [23,26-29]. By selecting different combinations of building blocks, the diacid/diol parts of the Arg-PEA repeating unit could be precisely controlled and adjusted, thus resulting in different structures and properties. In this study, the chemical structure of Arg-PEAs could be simply tuned by changing the number of CH_2 groups in the diacid/diol segment (x and y), salt type (p-toluenesulfonic acid salt or chlorine salt), and side group in the diacid/diol segment (Scheme 1).

The NP complex between this new series of Arg-PEA with nucleic acid was then formulated for the study on the relationship between chemical structure and polymer/NP properties. The physicochemical property of Arg-PEAs and the Arg-PEA/nucleic acid NP complex was evaluated and compared with an established quantitative index for the elucidation of the structure–property relationship (Scheme 1).

2. Experimental section

2.1. Synthesis and characterization of monomers and polymers

According the reported studies [23,24], the general scheme of Arg-PEA synthesis was divided into three major steps (Fig. 1). The detailed procedures are given in Supporting Information (SI).

The Arg-PEAs and monomers were characterized by various standard methods, and their structures were confirmed by nuclear magnetic resonance spectroscopy (400 MHz ^{1}HNMR, Bruker AM-400). The solubility of Arg-PEAs was evaluated by adding the Arg-PEAs into distilled water constantly until it precipitated at room temperature (25 °C).

2.2. Formulation of the Arg-PEA/nucleic acid NP complex

Three types of nucleic acids were used in this study: two DNAs (luciferase-encoding reporter plasmid COL (-77 2)/LUC and green fluorescence protein–encoding reporter plasmid DNA (GFP)) and one siRNA (Luc siRNA) and CpG (CpG and non-CpG). The Arg-PEA/nucleic acid NP complex was prepared by adding the nucleic acid solution into the Arg-PEA phosphate-buffered saline (PBS) solution with constant shaking to have a predetermined Arg-PEA-to-nucleic acid weight ratio (WR). In this paper, a wide range of WR (from 50 to 12,000) of Arg-PEA to nucleic acid was tested. The mixed solution was slightly vortexed for a few seconds and then equilibrated at room temperature for half an hour. All the Arg-PEA solutions and Arg-PEA/nucleic acid NP complexes were freshly prepared and used within 4 h.

2.3. Zeta potential and particle size of the Arg-PEA/nucleic acid NP complex

The particle size and zeta potential of the Arg-PEA/nucleic acid NP complex were characterized by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments) under room temperature. The morphology of the Arg-PEA/nucleic acid NP complex was observed under a JEM-1400plus transmission electron microscope (JEOL Ltd, Japan); phosphotungstic acid (1%) was used for the negative staining of NPs to enhance their electron contrast. Each type of Arg-PEA was measured with a series of WRs of Arg-PEA to Nucleic Acid. Each sample was measured in triplicate.

2.4. Cytotoxicity evaluation of the Arg-PEA/nucleic acid NP complex

The cells (SMC A10 cells, primary cells of the rat aortic smooth muscle cells [RSMCs], and Luc-HeLa cells) were seeded at 5,000 cells/well density in 96-well plates and cultured under 5% CO_2 at 37 °C for 24 h. Then, the SMC A10 cells and primary cells of RSMCs were treated with various Arg-PEA/DNA NP complex solutions for 4 h, and the Luc-HeLa cells were treated with the Arg-PEA/siRNA NP complex for 4 h and 24 h. the concentration of plasmid DNA and siRNA is 1 μg/ml and 1 nmol/ml, respectively. The medium was removed, and it was replaced by a complete DMEM medium. Cells treated with culture media were used as the negative control (NC). Further, cells treated with Arg-PEAs, Superfect®/nucleic acid and Lipofectamine 2000/nucleic acid complex were used as the positive control. After 48 h of incubation at 37 °C and 5% CO_2, the cell viability was evaluated by the MTT assay. Absorbance at 570 nm was measured using a microplate reader (BioTek Synergy4). The survival rate was calculated by recording the absorbance as a percentage relative to untreated cells.

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