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The effect of side-chain functionality and hydrophobicity on the gene delivery capabilities of cationic helical polypeptides

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ABSTRACT

The rational design of effective and safe non-viral gene vectors is largely dependent on the understanding of the structure–property relationship. We herein report the design of a new series of cationic, α-helical polypeptides with different side charged groups (amine and guanidine) and hydrophobicity, and mechanistically unraveled the effect of polypeptide structure on the gene delivery capability. Guanidine-containing polypeptides displayed superior membrane activities to their amine-containing analogues via the pore formation mechanism, and thus possessed notably higher transfection efficiencies. Elongating the hydrophobic side chain also potentiated the membrane activities of the polypeptides, while at the meantime caused higher cytotoxicities. Upon an optimal balance between membrane activity and cytotoxicity, maximal transfection efficiency was achieved which outperformed commercial reagent Lipofectamine™ 2000 (LPF2000) by 3–6 folds. This study thus provides mechanistic insights into the rational design of non-viral gene delivery vectors, and the best-performing materials identified also serve as a promising addition to the existing systems.

1. Introduction

The advance of molecular biology and genetic engineering has identified many disease-associated genes and their molecular regulators which provide potential targets for disease treatment. Gene therapy, mediated by the delivery of generic materials into target cells to promote or rectify the expression of specific gene, is a promising clinical modality to treat various human diseases, including cancer, infectious diseases, and immunodeficiency [1–4]. The key challenge towards gene therapy is the development of effective yet biocompatible delivery methods or vectors. Viral vectors, although highly efficient, often suffer from severe safety concerns such as carcinogenicity, immunogenicity and insertional mutagenesis [5]. Non-viral vectors, exemplified by cationic lipids and polymers, possess desired biocompatibility and minimal mutagenesis, and thus serve as desired alternatives to viral vectors for gene delivery [6] (Scheme 1).

Cell penetrating peptides (CPPs) are sequence-specific oligopeptides with distinguished membrane penetrating properties. A large number of CPPs, such as Pep-1, MPG, TP10, and melittin, adopt inherent helical structures or form helices in the cell membranes. Mechanistic simulation also unravels that the formation of a transmembrane helix presents a rigid amphiphilic structure to stabilize the membrane interactions and promote the membrane permeation [7,8]. Because of their desired membrane permeability, CPPs are able to facilitate the intracellular delivery of various cargos, such as proteins, peptides, nucleic acids, metals, and even nanoparticles. However, when used as gene delivery vectors, CPPs are often too short (fewer than 25 amino acid residues) and lack sufficient cationic charge density. Therefore, they are often unable to function as stand-alone vectors to independently condense and deliver genes, and in most cases, they were incorporated or conjugated to existing delivery vehicles as membrane-active ligands to enhance the cellular internalization and endosomal escape of the gene cargo [9–11]. In comparison, polypeptides with sufficient backbone length, such as poly-L-lysine (PLL) and poly-L-arginine (PLR), can independently condense and deliver genes, while the gene transfection efficiency remains low [12]. This is mainly because they adopt random coil conformation in the aqueous solution or when associated with phospholipid membranes due to the strong side chain charge repulsion, which thus greatly compromised the membrane activities of these high MW polypeptides [13].

To address the drawbacks of both short CPPs and polypeptides toward gene transfer, we recently developed a strategy to stabilize the helical structure of polypeptides by maintaining a minimum separation of 11-σ bond between the polypeptide backbone and the side charged groups, such that the side-chain charge repulsion

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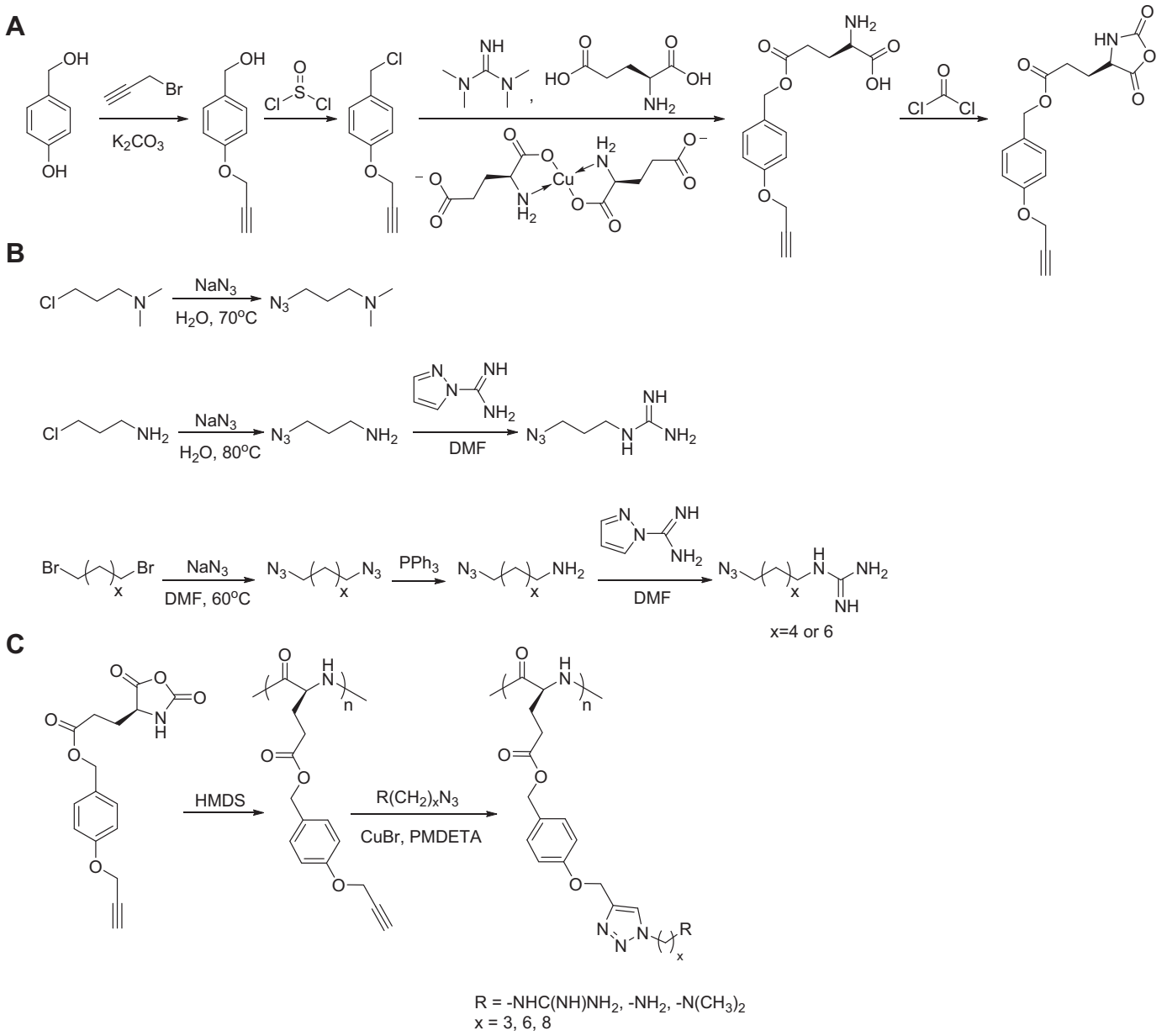

Scheme 1. Synthetic routes of γ-(4-propargyloxybenzyl)-L-glutamic acid based *N*-carboxyanhydride (POB-L-Glu-NCA) (A), azido amines/guanidines (B), and amine/guanidine functionalized polypeptides (C).

can be minimized and the helical structure can be stabilized [14]. A library of cationic polypeptides containing different amine side groups was thus synthesized and screening for their gene delivery capabilities. PVBLG-8 was identified to be top-performing material which notably outperformed traditional CPPs and polypeptides [15]. Although such screening process allows the identification of desired candidates, rational design over the polymer structure and mechanistic study on the structure–property relationship would render additional functionalities and features to maximize the gene delivery efficiency [16–21].

Arginine (Arg) residues are often rich in the primary structures of CPPs, and the guanidine groups of the Arg residues are crucial to the penetration efficiencies of CPPs because of their interactions with the sulfate groups of glycosaminoglycans localized on cell membranes [22]. The penetrating efficiency of the guanidine-rich CPPs can also be activated by hydrophobic counterions that complex around the guanidine-rich backbone to coat the highly cationic structure with lipophilic moieties and thus facilitate the membrane translocation. This also holds true for other synthetic polymers where incorporation of optimal hydrophobicity often leads to enhanced membrane activities [23,24]. Motivated by these understandings, we herein report our efforts in developing a new series of cationic, α-helical polypeptides with different side charged groups (amine and guanidine) and hydrophobicity, attempting to elucidate the effect of polymer structure and functionality on the gene delivery efficiency. We hypothesized that the incorporation of helical structures, guanidine groups, and elongated hydrophobic side chains would endow the polypeptides with considerable advantages over traditional CPPs and polypeptides, and an optimal combination thereof would thus lead to the maximization of the