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DNA delivery with hyperbranched polylysine: A comparative study with linear and dendritic polylysine

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ABSTRACT

PEI and polylysine are among the most investigated synthetic polymeric carriers for DNA delivery. Apart from their practical use, these 2 classes of polymers are also of interest from a fundamental point of view as they both can be prepared in different architectures (linear and branched/dendritic) and in a wide range of molecular weights, which is attractive to establish basic structure–activity relationships. This manuscript reports the results of an extensive study on the influence of molecular weight and architecture of a library of polylysine variants that includes linear, dendritic and hyperbranched polylysine. Hyperbranched polylysine is a new polylysine-based carrier that is structurally related to dendritic polylysine but possesses a randomly branched structure. Hyperbranched polylysine is attractive as it can be prepared in a one-step process on a large scale. The performance of these 3 classes of polylysine analogs was evaluated by assessing eGFP and IgG production in transient gene expression experiments with CHO DG44 cells, which revealed that protein production generally increased with increasing molecular weight and that at comparable molecular weight, the hyperbranched analogs were superior as compared to the dendritic and linear polylysines. To understand the differences between the gene delivery properties of the hyperbranched polylysine analogs on the one hand and the dendritic and linear polylysines on the other hand, the uptake and trafficking of the corresponding polyplexes were investigated. These experiments allowed us to identify (i) polyplex–external cell membrane binding, (ii) free, unbound polylysine coexisting with polyplexes as well as (iii) polymer buffer capacity as three possible factors that may contribute to the superior transfection properties of the hyperbranched polylysines as compared to their linear and dendritic analogs. Altogether, the results of this study indicate that hyperbranched polylysine is an interesting, alternative synthetic gene carrier. Hyperbranched polylysine can be produced at low costs and in large quantities, is partially biodegradable, which may help to prevent cumulative cytotoxicity, and possesses transfection properties that can approach those of PEI.

1. Introduction

Approaches that allow an efficient delivery of exogenous nucleic acids into cells are important tools, not only in fundamental research, but also in medicine and biotechnology. In experimental cell and molecular biology, for example, DNA delivery allows studying the expression, function and regulation of genes as well as protein function [1]. DNA delivery has also attracted widespread interest as a potential means to treat various inherited or acquired diseases [2,3]. Finally, efficient DNA delivery also forms the basis for the high yield production of recombinant proteins for preclinical studies or for investigations in structural biology [4,5].

DNA delivery can be mediated by viral and nonviral carriers. Viral carriers generally possess good transfection properties; however, the difficulties to produce these delivery agents on a large scale together with their immunogenicity have stimulated an increasing interest in the development of nonviral delivery systems [6,7]. Although a broad variety of non-viral gene carriers has been developed, the fundamental understanding of the basic structure–activity relationships that dictate the transfection efficiencies of these systems is still limited [8,9].

For two of the most extensively investigated classes of non-viral, synthetic polymer-based gene carriers, viz. polyethyleneimine (PEI) and polylysine, several studies have been published that report the influence of polymer architecture or molecular weight on transfection properties. In case of PEI, for example, several reports have shown that branched PEI is a less efficient carrier as compared to linear PEI of the same molecular weight [10–12]. Other reports, in contrast, have described no significant differences in gene expression efficiency between linear and

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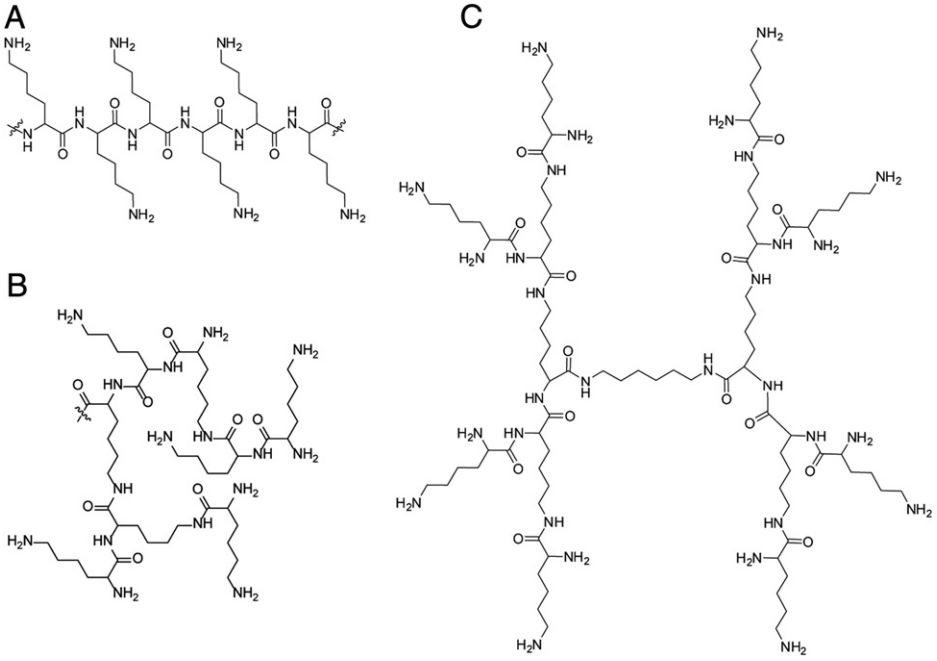
branched PEI analogs of comparable molecular weight [13]. Upon comparing dendritic and linear polylysine of similar molecular weight, Yamagata et al. found that gene expression mediated by the dendritic carrier was 100-fold higher than that of the linear analog [14]. Mannisto et al., in contrast, reported linear polylysine to have superior transfection properties as compared to dendritic polylysine [15]. In this latter study, however, the dendritic and linear polylysine carriers were not of comparable molecular weight. For both PEI and polylysine, transfection efficiencies generally have been found to increase with increasing molecular weight (or generation, in case of dendritic gene carriers) [15–20]. While the results from the studies cited above do provide some first guidelines for the development of novel polymer-based non-viral gene carriers, a profound understanding of the influence of polymer molecular weight, architecture and chemical composition on transfection efficiency would greatly facilitate the rational design of novel efficient DNA delivery systems.

This manuscript presents the results of a comparative study on the transfection properties of a library of linear (LPL), dendritic (DPL) and hyperbranched polylysine (HBPL) analogs (Scheme 1). HBPL is structurally related to DPL. However, whereas DPL is perfectly monodisperse and has a defect-free branched structure, HBPL is characterized by a broad molecular weight distribution and possesses a randomly branched architecture [21]. HBPL is an attractive alternative to DPL as it is easily prepared on a large scale in a one-step process, whereas DPLs are only accessible via a laborious multistep process [16]. For each of the 3 classes of polylysine analogs, a series of samples with different molecular weights was prepared in order to study the influence of molecular weight and architecture on the transfection efficiencies of these carriers. To assess and compare the transfection efficiencies of the LPL, DPL and HBPL analogs, the feasibility of these polymers to mediate the recombinant production of proteins via transient gene expression (TGE) was studied. TGE involves the introduction of an exogeneous nucleic acid into a cell without its stable integration in the host cell genome [22]. The transfection experiments that were carried out in this study were performed using Chinese hamster ovary (CHO) DG44 cells, which is a dihydrofolate reductase deficient subclone of the parental CHO cell line and frequently used for stable and transient gene expression [23,24]. In previous, preliminary experiments, it was found that HBPL has transfection properties that are comparable to those of similar molecular weight PEI, but leads to improved long-term (24 h) cell viability during transfection [19]. The increased cell viability was attributed to the partial biodegradability of HBPL, which, combined with the fact that it can be produced at low costs on a large scale, makes HBPL an interesting potential alternative to PEI. The present study builds upon these experiments and attempts to (i) obtain a better understanding of the transfection properties of HBPL and (ii) by comparison of HBPL with LPL and DPL establish first structure–property relations that allow the correlation of polycation molecular weight and architecture with transfection behavior. The first part of this manuscript will discuss the transfection properties of a library of LPL, DPL and HBPL analogs. These transfection experiments were followed by a series of experiments that concentrated on the internalization and intracellular trafficking of plasmid complexes with HBPL carriers, which are discussed in the second part of this manuscript.

2. Materials and methods

2.1. Materials

5-(and 6)-Carboxytetramethylrhodamine succinimidyl ester was obtained from Thermo Fisher Scientific (Lausanne, Switzerland). Cytochalasin D, nocodazole, chloroquine diphosphate salt and bafilomycin A were obtained from Sigma-Aldrich (Buchs, Switzerland). Paraformaldehyde (PFA) was obtained as a solution in water (Sigma Aldrich) and was used as received. DRAQ 5 was obtained from Biostatus Ltd. (Leicestershire, UK) and used according to the manufacturer's protocol. SnakeSkin Dialysis Tubing (molecular weight cut-off 3 kDa) was obtained from Thermo Fisher Scientific (Lausanne, Switzerland) and Sephadex G-25 and G-50 gel filtration media from GE HealthCare Europe GmbH (Glattbrugg, Switzerland). Dimethylformamide (DMF) was dried by passing over two columns containing molecular sieves using a Pure Solv™ 400 solvent purification system. Ultrahigh quality water with a resistivity of 18.2 MΩ·cm (at 25 °C) was obtained from a Millipore Milli-Q gradient machine equipped with a UV lamp and 0.22 μm filter. Aqueous solutions of linear polyethyleneimine (PEI) (Polysciences, Eppenheim, Germany) were prepared from a powder at a concentration of 1 $mg \cdot mL^{-1}$ with the pH adjusted to 7 with 0.1 M HCl. Prior to use these solutions were sterilized by filtration with a 0.22 μm



Scheme 1. Schematic representation of the structure of (A) linear polylysine (LPL); (B) hyperbranched polylysine (HBPL) and (C) a 3rd generation dendritic polylysine (DPL).