



Pharmaceutical nanotechnology

Zeta potential changing phosphorylated nanocomplexes for pDNA delivery



Sonja Bonengel, Felix Prüfert, Max Jelkmann, Andreas Bernkop-Schnürch*

Center for Chemistry and Biomedicine, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria

ARTICLE INFO

Article history:

Received 25 June 2015

Accepted 4 October 2015

Available online 2 April 2016

Keywords:

Zeta potential changing systems

pDNA

Polyethyleneimine

Phosphatase

6-

Phosphogluconic acid

ABSTRACT

The objective of this study was to evaluate the suitability of a zeta potential changing system as gene delivery system. The phosphate ester bearing ligand 6-phosphogluconic acid (6-PGA) was attached to linear and branched polyethyleneimine (PEI) via a carbodiimide-mediated reaction whereby 287 μmol and 413 μmol 6-PGA could be coupled per gram polymer. Nanocomplexes of these modified polymers with pDNA showed a zeta potential of +12 mV for nanocomplexes with the linear PEI-6PGA and +16 mV in case of the branched derivative. By the addition of carboxymethylcellulose (CMC), zeta potentials of the complexes were reduced to +2.86 and +3.25, respectively. Phosphate release studies on Caco 2 cells and HEK-293 cells demonstrated the ability to cleave the phosphate ester. Compared to HEK-293 cells, enzymatic degradation of the phosphate ester in Caco 2 cells was 2.3-fold higher from nanocomplexes comprising linear PEI and 4.3-fold higher from those with branched PEI. Furthermore, incubation with alkaline phosphatase led to an increase in the zeta potential of nanocomplexes based on linear PEI-6PGA to +6.96 mV and +8.26 mV in nanocomplexes comprising branched PEI-6PGA. Studying transfection efficiency in Caco 2 cells and HEK-293 cells, a higher expression of the green fluorescent protein (GFP) could be detected in HEK-293 cells. In presence of a phosphate inhibitor, transfection efficiencies were decreased in both cells lines, due to a lacking shift of the zeta potential of the tested pDNA complexes. According to these results, zeta potential changing systems seem to be a promising strategy for future gene delivery systems, as this concept allows the in situ generation of positive charges in close proximity to the cellular surface.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Within the field of gene delivery, new gene vectors are actively researched aiming at DNA delivery systems with a better passage of endogenous barriers, such as the membrane barrier, the mucus barrier and the enzymatic barrier. Due to a lower immunogenicity, the primary focus is on non-viral vectors, such as cationic liposomes and cationic polymers. Forming tightly condensed complexes (Boussif et al., 1995) with DNA and protecting the DNA against enzymatic degradation via DNases (Moret et al., 2001) while promoting efficient cellular uptake, polyethyleneimine (PEI) emerged as an efficient alternative to the rather expensive liposomes and other commercially available transfection reagents. Although, the use of PEI as transfection reagent is limited, as transfection efficiency of PEI was found to be significantly lowered

in the presence of serum (Chollet et al., 2002) or in mucus secreting cells (Florea et al., 2002), due to electrostatic interactions with negatively charged proteins or mucus components. These adsorptive effects can be minimized by reducing the positive net charge of PEI complexes, but thereby transfection efficiency is decreased as well, as the positive charge is essential for the intracellular uptake. Recently, however, the novel concept of zeta potential changing nanoparticles could be shown as a promising strategy to combine advantageous properties linked to opposite surface charges. Hereby, a shift of the zeta potential can be achieved after the removal of a negatively charged moiety via enzymatic degradation. So far, this strategy could be successfully applied for nanoparticles bearing phosphate ester substructures and a proof of principle is provided for nanoparticles comprising 6-phosphogluconic acid (6-PGA) modified PEI. Via coupling of 6-PGA to the polymer, a substrate of the brush border membrane-bound enzyme intestinal alkaline phosphatase (IAP) was introduced and particles were prepared with carboxymethylcellulose (CMC). Enzymatic degradation of the phosphate ester and subsequent elimination of negatively charged phosphate ions led to a switch of the zeta

* Corresponding author. Fax: +43 512 507 58699.

E-mail addresses: andreas.bernkop@uibk.ac.at, a.bernkop@thiomatrix.com (A. Bernkop-Schnürch).

potential from -6 mV to $+3$ mV (Bonengel et al., 2015). As this strategy allows the in situ generation of positive charges required for cellular uptake near the cell surface, zeta potential changing systems might be an interesting concept for DNA delivery systems.

Accordingly, this study was aimed to apply the concept of zeta potential changing nanoparticles in the field of gene delivery. Therefore, 6-phosphogluconic acid was coupled to linear and branched PEI (Fig. 1), respectively, which were used for the preparation of nanocomplexes containing a model plasmid encoding for green fluorescent protein (GFP). These nanocomplexes were characterized in terms of size, zeta potential and toxicity profile. Furthermore, the influence on transfection efficiency of these nanocomplexes with pDNA was investigated in Caco 2 cells and HEK-293 cells.

2. Materials and methods

2.1. Materials

Polyethyleneimine (PEI-bran), branched (Mwt 50,000–100,000 Da), 30% (w/w) aqueous solution and linear polyethyleneimine (PEI-lin) (Mwt 60 000 Da), 50% aqueous solution were received from Alfa Aesar GmbH & Co., KG, Karlsruhe, Germany. Agarose, agar-agar and LB-medium were purchased from Carl Roth, Karlsruhe, Germany. The plasmid extraction kit was received from Qiagen, Hilden, Germany and Lipofectamine® 2000 from Life Technologies, Carlsbad, CA, USA. The green fluorescent plasmid was amplified in *Escherichia coli*, which were supplied by Addgene (pcDNA3-EGFP, Addgene plasmid 13031). 1-Ethyl-3-(3-

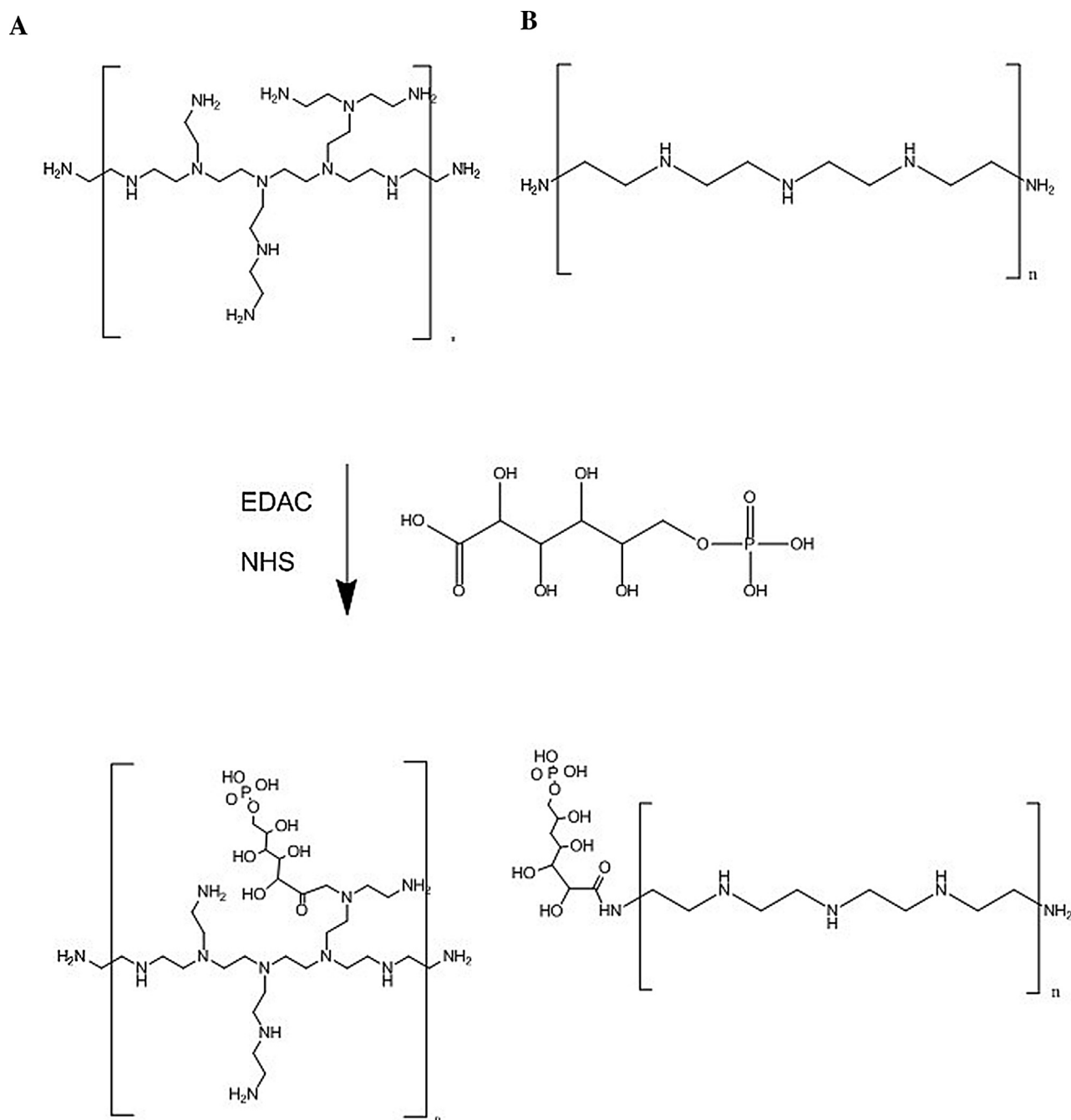


Fig. 1. Synthesis of PEI-6PGA.

6-PGA was covalently attached to the polymeric backbone of branched PEI (A) and linear PEI (B) via amide bond formation mediated by a carbodiimide.

Download English Version:

<https://daneshyari.com/en/article/2500911>

Download Persian Version:

<https://daneshyari.com/article/2500911>

[Daneshyari.com](https://daneshyari.com)