STRUCTURALLY WELL-DEFINED COPOLYMERS OF POLY(ETHYLENE GLYCOL) AND LOW MOLECULAR WEIGHT LINEAR POLYETHYLENIMINE AS VECTORS FOR GENE DELIVERY

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Summary

Structurally well-defined ABA triblock copolymers with low molecular weight ***linear*** polyethylenimine (PEI) as A block and poly(ethylene glycol) (PEG) as B block have been prepared and used for gene delivery. PEI–PEG–PEI 2100–3400–2100 can effectively condense DNA, giving small-sized polyplexes (<100 nm) with reduced zeta-potentials (<10 mV). Initial experiments show that these polyplexes have a better transfection efficiency and a decreased cytotoxicity compared to polyplexes based on low molecular weight linear PEI polymers.

Introduction

Gene therapy holds great promise for treating life-threatening diseases like cancer [1,2]. In the past decade, nonviral vectors especially cationic polymeric systems have attracted growing interest since they offer many advantages over the viral counterparts such as ease of production, low immunogenicity, and great flexibility with regard to vector modification and DNA incorporation. Poly(L-lysine) (PLL), polyethylenimine (PEI), poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), and polyamidoamine (PAMAM) dendrimers are among the most widely studied polymers for gene delivery [3]. All these polymers have nevertheless shown in vivo toxicity, which has become one of the major limiting factors for their advance to the clinical uses. For example, PEI polymers were regarded as promising nonviral gene carriers due to their superior transfection efficiency, the in vivo administration of PEI/DNA complexes into mice was, however, reported to give severe toxicity [4].

The aim of this study was to design safer polymeric gene delivery systems, for which copolymers based on poly(ethylene glycol) (PEG) and low molecular weight linear PEI were synthesized. The preliminary in vitro experiments show that the polyplexes of such copolymer have a low toxicity and are able to transfect COS-7 cells.

Experimental methods

Polymer Synthesis

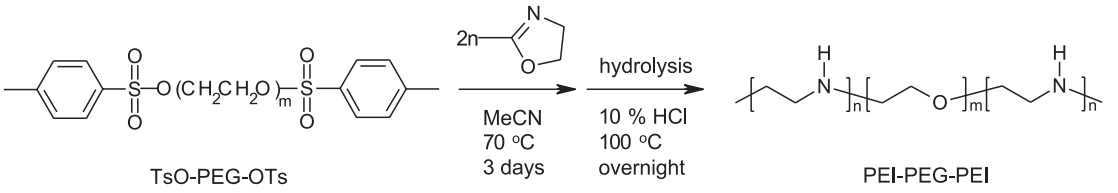
PEI-PEG-PEI triblock copolymers were prepared from cationic polymerization of 2-methyl-2-oxazoline (MeOZO) using PEG-bis(tosylate) as a macroinitiator followed by hydrolysis. The polymerization was conducted in CH_3CN at 70 °C for 3 days, which led to a quantitative yield of PMeOZO–PEG–PMeOZO copolymers with prescribed compositions. The hydrolysis of PMeOZO–PEG–PMeOZO was carried out in 10 wt.% HCl solutions overnight at 100 °C. A low molecular weight linear PEI polymer has been synthesized in an analogous way except using methyl tosylate instead of PEG-bis(tosylate).

In vitro transfection and cell viability assay

Transfection experiments were performed with COS-7 cells by using the plasmid pCMV-LacZ as reporter gene as reported previously for PDMAEMA [5]. Two parallel transfection series, one for the determination of reporter gene expression (β-galactosidase) using ONPG assay and the other for the evaluation of cell viability by XTT assay, were carried out in separate 96-well plates. PDMAEMA/DNA complex with a ratio of 3:1 (w/w) was used as a reference for gene transfection efficiency.

Results and discussion

ABA triblock copolymers with low molecular weight ***linear*** polyethylenimine (PEI) as A block and poly(ethylene glycol) (PEG) as B block were synthesized by cationic polymerization of 2-methyl-2-oxazoline using PEG-bis(tosylate) macroinitiator followed by acid hydrolysis (scheme 1). NMR and GPC results showed that all copolymers have controlled compositions and molecular weights. An initial in vitro transfection experiment revealed that low molecular weight linear PEI polymer with an Mn=100 (denoted as PEI 2100) has low toxicity and is not able to transfer DNA into COS-7 cells, despite the fact that PEI 2100 can complex DNA into small-sized



Scheme 1. Synthesis of polyethylenimine-b-poly(ethylene glycol)-b-polyethylenimine (PEI–PEG–PEI) triblock copolymers.

nanoparticles (80~100 nm) when PEI 2100/DNA ratio is above 1:1 (w/w). In contrast, the triblock copolymer, PEI–PEG–PEI with Mn (PEI)=2100 and Mn (PEG)=3400 (denoted as PEI–PEG–PEI 2100–3400–2100), displayed marked transfection activity. The transfection efficiency increased with increasing polymer/DNA ratios, in which a transfection efficiency of 25% relative to PDMAEMA was achieved at a polymer/DNA ratio of 96:1 (w/w) (Fig. 1). The addition of endosomal escape peptide INF-7 did not improve the transfection efficiency, indicating that the PEI–PEG–PEI triblock copolymers are well capable of disrupting endosomes. Importantly, this triblock copolymer has a substantial lower cytotoxicity than low molecular weight PEI polymer (Fig. 2). Biophysical characterizations revealed that PEI–PEG–PEI 2100–3400–2100 effectively condenses DNA at a polymer/DNA ratio of 3:1 (w/w), giving polyplexes with sizes less than 100 nm and reduced zeta-potentials (<10 mV). The low zeta-potential of these polyplexes is likely due to the shielding of the surface charge of the polyplexes by PEG chains and this may account for the low toxicity of the triblock copolymer. It should be noted that in the presence of serum, both PEI 2100 and PEI–PEG–PEI 2100–3400–2100 are essentially nontoxic at polymer/DNA ratios up to 96:1 (w/w).

Although the transfection efficiency of PEI–PEG–PEI 2100–3400–2100 is low compared to high molecular weight PEI and PDMAEMA, this triblock copolymer has the apparent advantage of its low toxicity. These first results have demonstrated that it is possible to markedly enhance the transfection efficiency and meanwhile decrease cytotoxicity of low molecular weight linear PEI by conjugating to PEG. Currently, copolymers with different macromolecular architectures like star-shaped and multi-block copolymers of PEG and low molecular weight linear PEI are under investigation. The relationship between polymer structures and their biological properties, particularly transfection activity and cytotoxicity, will be studied.

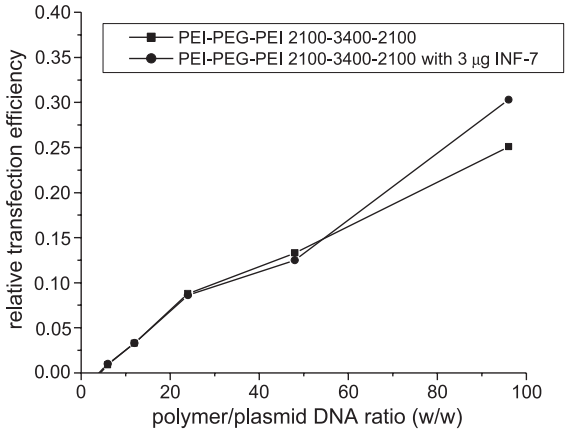


Fig. 1. Transfection activity of PEI–PEG–PEI 2100–3400–2100/plasmid DNA Complexes in COS-7 cells as a function of polymer/DNA ratios. Transfection data are relative to PDMAEMA/plasmid DNA at 3:1 ratio (w/w).

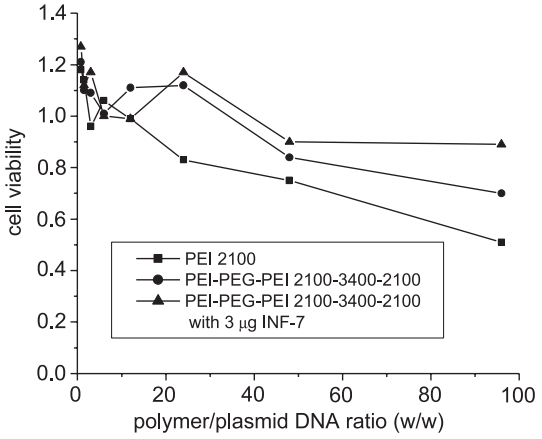


Fig. 2. The viability of COS-7 cells incubated with polyplexes prepared at different polymer/plasmid ratios measured by the XTT assay.

Conclusion

We have demonstrated that copolymers based on PEG and low molecular weight linear PEI can efficiently condense DNA into nanoparticles. These polyplexes have markedly improved transfection efficiency and reduced cytotoxicity as compared to the polyplexes of low molecular weight linear PEI polymers. In the future, the macromolecular structure and the molecular weights of the different blocks of the copolymers will be varied to achieve optimal transfection efficiency, which may eventually lead to promising vectors for gene delivery.

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