Telomerization Increases Transfection Efficiency in New Polymeric Gene Vectors Derived from Pyrrolidine

D. Velasco¹, E. Collin², J. San Roman¹, A. Pandit², C. Elvira¹

Corresponding Author: diegovb@ictp.csic.es

¹Biomaterials Department, Institute of polymer science & Technology, CSIC, Juan de la Cierva,3, 28006, Madrid, Spain.
²National Centre for Biomedical Engineering Science (NCBES), National University of Ireland, Galway, Ireland.

Introduction

Virus vectors have been widely used as gene carriers because of the high efficiency of these vectors delivering DNA. However, toxicity and immunogenic effects have promoted research in alternative vectors such as cationic polypeptides, lipids and polymers. These synthetic molecules have lower transfection efficiency than viruses but a better safety, greater flexibility and easy production. We have developed a new cationic polymer (tertiary amine group in its structure), poly (N-ethylpyrrolidine methacrylamide), poly-EPA¹ and copolymers with dimethyl acrylamide (DMA), as potential gene vectors. In order to improve the transfection efficiency of this cationic polymer (poly-EPA), primary amines were incorporated at the end of the macromolecular chains by telomerization reaction.

Materials and Methods

The homopolymer, copolymers poly (EPA-co-DMA) and telomers were prepared by radical polymerization and telomerization reactions in solution, characterized by ¹H NMR, size exclusion chromatography (SEC), differential scanning calorimetry (DSC), acid-base titration and ninhydrin assay. The polynplexes were characterized by transmission electron microscopy (TEM), agarose gel electrophoresis, coulter, and zeta potential. Rat 3T3 fibroblasts were used to test the toxicity and transfection. Guangia luciferase assay kit was used for the transfection studies to quantify the luciferase protein in the cell media extract. Alamar Blue and PicoGreen assays were used to evaluate the toxicity of the polymers and polynplexes. Blood compatibility studies were carried out with the telomers.

Results

Polymers exhibited ionization at physiological pH and were found to complex DNA by electrophoresis analysis. All telomers exhibited higher ionization degree at physiological pH. An average size from 100 to 400nm and surface charge of the electrostatic polynplexes from 10 to 20mV were observed. Complexes presented a shape circular and rods for the copolymers and telomers respectively. In the transfection studies luminiscence emission was observed in the homopolymer and copolymers with values lower than 2-3 times than poly-lysine (control) whereas in the case of telomers increased 2-5 times respect to poly-lysine. Cell viability, cell proliferation and blood compatibility showed no difference compared to controls.

Discussion and Conclusions

By controlling the amount of cisteamine in the telomerization reaction it was possible to obtain telomers with different molecular weights and therefore with different concentration of –NH₂ groups. Telomers were found to be non-toxic, and they increases the transfection efficiency up to 5 times respect to poly-lysine control.

References


Acknowledgments

Funding by Proof of Concept Enterprise Ireland and by MAT2007-63355 (CICYT-Spain).

Disclosures

Authors have nothing to disclose.