Study of the colloidal stability of DNA/polycation complexes in presence of biological polyanions

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Natural or synthetic polycations are used in nucleic acid-based therapies (new mRNA vaccines or gene therapy) as complexing agents which interact electrostatically with nucleic acids, condense them into nanoparticles, protect them and control their entry into cells [1]. However, although the formation of particles known as complexes is well documented [2], few studies are focused on the physical chemistry behind the disassembly after the endosomal escape, especially under physicochemical conditions found in an intracellular environment. There are several theories of the disassembly of these complexes, one of them consisting in the exchange between the polycations of these particles with competitive biological polyanions [3].

This research project focuses, first, on the study of the complexation mechanism of two polycations, *i.e.* chitosan (CS) and branched polyethyleneimine (PEI-b) with nucleic acids (calf-thymus DNA and pDNA). Then, the colloidal stability of the obtained complexes was studied in presence of biological polyanions by means of size evolution and aggregation through Dynamic Light Scattering (DLS) measurements. To accomplish these objectives, polycations (CS and PEI-b) and biological polyanions (hyaluronic acid, HA, chondroitin sulfate, ChS and Heparin, Hp) were characterized through ^{1}H NMR and Size Exclusion Chromatography (SEC) in order to determine their structure, molecular weight and dispersity. Then, the stoichiometry of polycation/polyanion complexes was determined through conductivity and □-potential measurements. Nucleic acid-based complexes size, polydispersity index, global charge and morphology were determined using DLS, ζ-potential measurements and Transmission Electron Microscopy (TEM), at different charge ratios (N^{+}/P^{-}: corresponding to the ratio between protonated amines from polycations and negatively charged phosphates from DNA). Complexes sizes were found to be between 100 nm and 250 nm, depending on the polycation nature and its molecular weight. Complexes global charge were found to be between 23 and 35 mV, depending on the polycation charge density. Moreover, they were found to be colloidally stable during at least 15 days after their formulation. The stability of these nucleic acid-based complexes in presence of biological competitive polyanions was studied through DLS measurements. It was found that the stability is dependent on polycation pH and on the charge ratio N^{+}/P^{-} of the complex. The aggregation point of nucleic acid-based complexes after the addition of a certain amount of biological competitive polyanion was determined using different polyanions. It was found to be dependent on the charge density of the polyanion.

References

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