Polyallylamine-plasmid complex: A physico chemical study

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Plant genetic engineering for phytoremediation approaches is still poorly explored due to difficulties in genetic manipulation of non-model-plant species, often used for this approach. A promising strategy for enhancing phytoremediation is to generate plants with enlarged root system and an increased absorption capacity, obtained by modifying genetic traits that regulate root development.

Conventional DNA delivery methods for plants transformation are time consuming and have important limitations such as host- species barriers and low transformation efficiency.

In plant genetic engineering, nanomaterials have been used as vehicles for the delivery of plasmid DNA, short-interfering RNA (siRNA) and proteins to plants through the infiltration of leaves. However, so far there are no works reported about the use of polymeric nanoparticles for generating non-food crop with enhanced abilities for phytoremediation purposes.

Furthermore, exogenous DNA and RNA delivery into plants is a great challenge because the cell wall provides an important barrier, limiting molecules diffusion inside the plant cells thereby reducing the efficiency of plant genetic engineering.

Recently, in animal cell studies, modified polyallylalamine hydrochloride (PAH) polymeric nanoparticles have found applications as vectors of genetic material. These cationic polymers interact electrostatically with negatively charged nucleic acids[1,2,3]. Among PA, polyallylamine phosphate (PAN) polymeric nanoparticles have been used successfully as vectors for siRNA. Nano polymer-siRNA complexes are stable at pH values between 7 and 9, while dissociation occurs at pH less than 6 or greater than 9. These complexes enter the cells by endocytosis and are directed to lysosomes where the acid pH induces the dissociation of the complexes, leading to the release of siRNAs in the cytoplasm[4].

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In this work we have studied the complexation of nucleic acids for plant genetic with PAH molecules with different modifications: oleic acid and dextran, aiming at having a more physical insight on the properties of the polyplexes formed. We have studied the process of complex formation and characterized the polyplex nanoparticles by a combination of techniques: Fluorescence Correlation Spectroscopy (FCS), Dynamic Light Scattering (DLS), Small Angle X-ray Scattering (SAXS) and Transmission Electron Microscopy (TEM).

FCS is used to study the changes in diffusion time of fluorescently labelled PAH after complexation. Stability of the polyplexes in relevant pH's is studied by FCS. Using PAH and nucleic acids both fluorescently labelled but with two different dyes with non-overlapping emission we have determined the actual stoichiometry of the complexes calculating the fraction of free polymer for different N/P ratios (amount of protonable amine groups per each phosphate group) considered for polyplex formation FCS data are complemented with TEM/DLS/SAXS

References

[1] Oskuee et al., 2014.
[2] Oskuee et al., 2015.
[3] Alekseenko et al., 2020.
[4] Andreozzi et al., 2017.

Figures

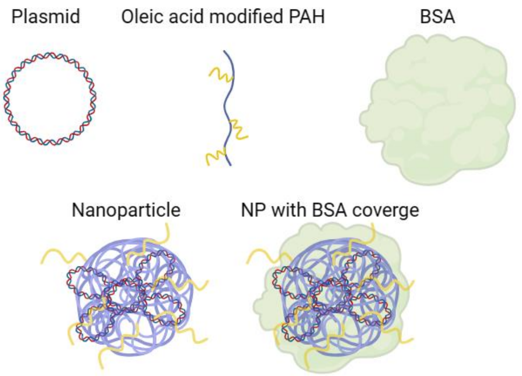

Figure 1. Scheme of the self-assembled nanoparticles with and without BSA coverage.

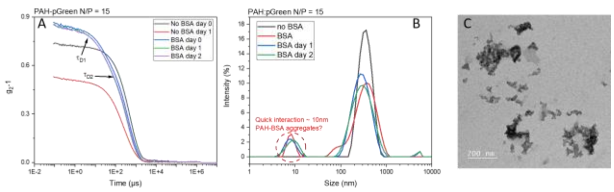

Figure 3. NP's with BSA at higher N/P ratio after 0, 1 and 2 days of incubation. A) Autocorrelation functions, B) size distributions by intensity obtained by DLS and C) TEM image of them.