Internalization and toxicity of amine and hydroxyl terminated poly(amidoamine) dendrimers to photosynthetic microorganisms

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Abstract

Poly(amidoamine) (PAMAM) dendrimers are hyper-branched polymers with uniform size, defined molecular weight, large internal cavities and a high number of surface groups that make them particularly suitable for a number of biomedical and technological applications [1]. It has been found that surface functionalization is the main factor modulating the toxicity of dendrimers to mammalian cells lines, but their effects for aquatic microorganisms is still largely unknown [2]. We are presenting results on the effect of generation G2, G3 and G4 amine-terminated ($–NH_2$) and hydroxyl terminated (-OH) PAMAM dendrimers towards the green alga *Chlamydomonas reindhartii* and the cyanobacterium *Anabaena* PCC7120.

We studied the internalization of dendrimers using PAMAM-Alexa Fluor 488 conjugates and their toxic effect by tracking the inhibition of growth rate and the formation of reactive oxygen species (ROS) revealed by the fluorescent dyes 2',7'-dichlorofluorescein diacetate and C4-BODIPY®. Fluorescence studies were performed by flow cytometry and confocal microscopy. Ultrastructure alterations were studied by transmission electron microscopy (TEM). We also report a physicochemical characterization of PAMAM dendrimers in culture media based on size distribution obtained using dynamic light scattering and zeta potential measured via electrophoretic light scattering.

All amine-terminated and the G4 hydroxyl-terminated dendrimers significantly inhibited the growth of both microorganisms, the effect on the green alga being higher. Expressing concentrations in terms of molarity, it was observed a higher toxicity for growing dendrimer generation. We also detected a hormetic effect for hydroxyl-terminated dendrimers at low concentrations. Cationic PAMAM dendrimers were largely and quickly internalized in both organisms showing a diffuse distribution in cyanobacteria and affecting mitochondria in algal cells.

All cationic dendrimers and G4-OH significantly increased the formation of ROS in both organisms. ROS formation was not related with the chloroplast or photosynthetic membranes. Significantly, photosystem II photochemistry was not affected. ROS damage resulted in cytoplasm disorganization and cell deformities and were associated in the green alga to lipid peroxidation in mitochondria. In the cyanobacterium we also observed intense ROS formation, cell wall and membrane disruption and loss of cytoplasmic contents.

These results warn against the generalization of the use of dendrimers which may pose significant risk for the environment and particularly for primary producers which are determinant for the health of natural ecosystems. Hydroxyl-functionalized molecules, still bearing a positive charge, have also been shown toxic for photosynthetic organisms.

References

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