PROTEOME ANALYSIS OF THE MICROALGAE CHLAMYDOMONAS REINHARDTII RESPONSES TO dsDNA-COATED GOLD NANOPARTICLES

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Introduction

Gold nanoparticles carrying dsDNA oligonucleotides have been applied in the development of novel spatiotemporal control of gene expression. However, the physiological effects of these carrier systems and silencing strategies at the proteome level, following cellular internalization and nucleic acid delivery, remain largely unknown. Therefore, we have conducted mass spectrometry-based proteomic analysis of *C. reinhardtii* cells transfected with gold nanoparticles (AuNPs) coated with dsDNA, revealing alterations in cell physiology.

Material and Method

C. reinhardtii CC503 cw92 mt+ cells (Chlamydomonas Resource Center, USA) were cultured at 100 µmol m?² s?¹ constant illumination under mixotrophic conditions. Cells (1×10^8) were harvested, washed with D-sorbitol solution, and incubated with AuNPs coated with sense and antisense DNA (DNA-switch) targeting the N-acetyl glutamate synthase (NAGS) gene. Cells incubated with TAP medium alone served as the control condition. After 1 hour of cell incubation with DNA-switch, the cells were harvested and total proteins extracted using Urea-Thiourea buffer and sonication. Protein concentration was determined by Bradford method, and 2 µg of total protein reduced, alkylated, and digested with Trypsin (Promega, Madison, WI, USA). Peptides were desalted and eluted in 0.1% formic acid and analyzed by mass spectrometry using an Orbitrap Fusion Lumos coupled to a nano LC–MS/MS (Thermo, USA). Proteins were identified using MaxQuant and Perseus software platform. Comparative proteome analysis was performed using Label-free quantification (LFQ) algorithm and proteome functional annotation analysis was performed using the ClueGO plugin in the CytoScape application. Statistical analysis was performed with significance of P-value<0.05.

Discussion

The statistical analysis revealed 160 protein groups differentially expressed between control and treatment. Of those protein groups, 40.5% of the respective protein identifiers (ID) were recognized by ClueGO, which attributed 12 proteins to the GO term GO:0006091 (precursor metabolites and energy), as well as 9 proteins to GO:0015979 (photosynthesis). Both biological processes can be affected by AuNPs' physical properties, since it could enhance the number of electrons available for transfer in photosynthetic machineries as well as the photon absorption through its plasmonic band. In the analysis, 9.72% proteins were attributed to electron transport chain biological processes. Also, 3 proteins were related to response to toxic substances (GO:0009636), although indicating detoxification response abilities.

Additionally, the oligonucleotides used were designed to match the sequence of the mRNA from NAGS gene transcript, involved in glutamine and regulation of lipids metabolic process, and our functional annotation revealed that 3.47% proteins annotated were related to neutral lipids accumulation. This could be explained by a small spontaneous release of antisense oligonucleotides during cells incubation, which induced the role of NAGS in the regulation of lipids accumulation in the cells.

Conclusions

Transfection has demonstrated to elicit physiological effects on the cells, affecting photosynthetic metabolism. The stress-related proteins identified did not indicate irreversible damage to the cells. Moreover, the dsDNA-coated AuNPs appear to influence metabolic pathways associated with photosynthetic efficiency and glutamate metabolism.

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