

AG-IONS PLAY THE MAIN ROLE IN SILVER NANOPARTICLES TOXICITY IN THE CILIATE *TETRAHYMENA THERMOPHILA*

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Silver nanoparticles (AgNPs) are highly toxic to aquatic organisms, however, there is no consensus whether the toxicity is caused solely by released Ag-ions or also by reactive oxygen species (ROS). Here, the effects of protein-coated AgNPs (14.6 nm, Collargol) were studied on viability, oxidative stress and gene expression levels in wild type strains (CU427 and CU428) of unicellular ciliate *Tetrahymena thermophila*. Viability-based 24 h EC₅₀ values of AgNPs were relatively high and significantly different for the two strains: ~100 mg/L and ~75 mg/L for CU427 and CU428, respectively. Similarly, the expression profiles of oxidative stress (OS) related genes in the two strains were different. However, even though some OS related genes were overexpressed in AgNP-exposed ciliates, intracellular ROS level was not elevated, possibly due to efficient cellular antioxidant defence mechanisms. Compared to OS related genes, metallothionein genes were upregulated at a considerably higher level (14 versus 5000-fold) suggesting that Ag-ion mediated toxicity mechanism of AgNPs prevailed over OS related pathway. Also, comparison between Ag-ions released from AgNPs at EC₅₀ concentration and the respective EC₅₀ values of AgNO₃ indicated that Ag-ions played a major role in the toxicity of AgNPs in *T. thermophila*. The study highlights the importance of combining physiological assays with gene expression analysis in elucidating the mechanisms of action of NPs to reveal subtle cellular responses that may not be detectable in bioassays. In addition, our data filled the gaps on the toxicity of AgNPs for environmentally relevant and abundant organisms. The parallel study of two wild type strains allowed us to draw conclusions on strain to strain variability in susceptibility to AgNPs.



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