

Assessing ionic silver availability to algae from differently coated silver nanoparticles

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Introduction

Because its biocide properties [1-4], silver nanoparticles (AgNP) are present in numerous consumer products. Toxicity of AgNP to organisms is related with both the formation of ionic silver (Ag^+) and interactions between AgNP and Ag^+ with cell membranes [1-3]. Hence, studies on AgNP toxicity are challenged by understanding the contribution of these causes to the whole toxicity [5]. Thus, characterization of AgNPs in toxicity experiments also requires determining the bioavailable concentration of ionic silver. At present methodological limitations result in under estimation of readily bioavailable ionic silver, especially if ionic silver is formed at nanoparticles-cell interface and is immediately taken up by cells [3]. In addition, nanotechnological development relies not only in nanomaterials synthesis but in functionalization of their surfaces, adding more complexity to these studies. In this case, the availability of ionic silver might be strongly determined by the chemistry of the different products used as a coating.

In this work we propose a method to examine the role of Ag^+ in explaining toxicity of differently coated AgNPs to algae, by using cysteine to assess Ag^+ bioavailability. We thus assessed the toxicity of AgNPs coated with 5 different chemicals, on the photosynthesis of *Chlamydomonas reinhardtii*. Experiments in presence of cysteine which is a strong Ag^+ ligand was used to estimate the amount of Ag^+ present in the exposure media. From our previous work on AgNP toxicity [3] we hypothesize: a) the importance of Ag^+ to explain the toxicity of AgNP and b) toxicity to photosynthesis might help us to assess the ionic silver readily bioavailability for algae. This approach allows us to estimate ionic silver bioavailable for algae, overcoming methodological limitations associated to the use of direct chemical analysis.

Results

Experiments were carried out using 5 different AgNP, coated with carbonate -CO₃-, polyethelene glycol -PEG-, lactate -LAC-, chitosan -CHI- and polyvinyl pyrrolidone -PVP-. Toxicity of AgNP to photosynthesis was assessed by concentration-response experiments exposing algae to increasing concentrations of the different AgNP (Tab. 1). Algal photosynthetic yield was measured over 1 hour exposure time by fluorometry [3].

AgNP	% of Ag^+	EC ₅₀ (μM Agtot)	EC ₅₀ (μM Ag^+)	μM Cys
CO ₃	1	2,98	0,030	0,405
PEG	16,2	1,29	0,209	0,420
LAC	9,2	2,15	0,199	0,510
CHI	3,7	2,84	0,106	0,500
PVP	20	0,78	0,154	0,491

Table 1. AgNP's EC₅₀ have been calculated as a function of the total Ag present in the suspensions, as a function of Ag^+ present (calculated using chemical analysis). Last column shows the amount of Cysteine required to completely abolish the toxicity of EC₅₀.

Based on total Ag concentration (Tab. 1), AgNP EC₅₀ ranged from 0,78 to 2,98 µM (Tab. 1). Cysteine completely abolished AgNP toxicity (previous experiments, data not shown), indicating that toxicity was mediated by Ag⁺ and at the same time that different coatings were not toxic to photosynthesis. Hence, it was expected that EC₅₀ values calculated as a function of the Ag⁺ present in the experimental suspensions (assessed using chemical analysis) would converge to similar values. But, these values (Tab.1) showed again a wide range of variation (from 0.03 to 0.2).

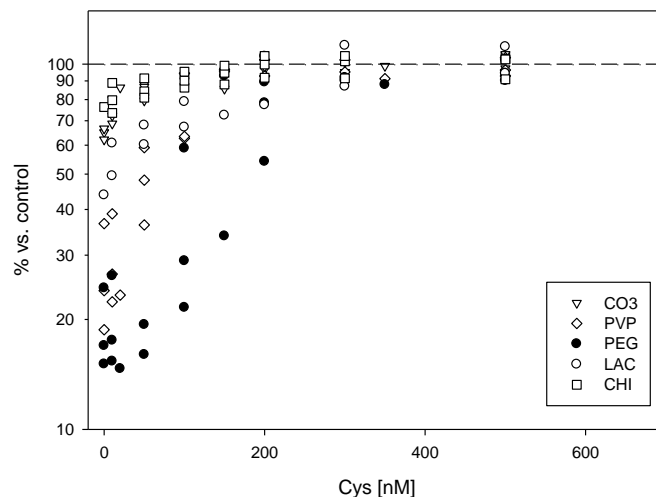


Figure 1. Effect of increasing cysteine concentrations on the toxicity of AgNPs EC₅₀. The cysteine required to abolish toxicity was calculated as the intersection of every curve with the 100% line.

Then, we considered the possibility that Ag⁺ concentration used to calculate EC₅₀ was underestimated (see introduction). We calculated the cysteine concentration required to completely abolish toxicity of AgNPs EC₅₀, as an estimate of bioavailable Ag⁺ (Fig.1). Cysteine concentrations needed to completely abolish AgNP toxicity showed similar values for all AgNP, regardless their coatings (from 0,405 to 0,500 µM). This experiment strongly suggested that Ag⁺ bioavailable to algae was in all cases around 0,4-0,5 µM (cysteine binds Ag⁺ in 1:1 stoichiometry).

Together, results indicate that chemical analyses are not enough to characterize exposure conditions. Use of cysteine and algal photosynthesis might be a helpful control method, especially when dissolved metals might be released or formed upon nanoparticles-cell interface interactions.

References

- [1] Pal, S., Y.K. Tak, and J.M. Song, *Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli*. Applied and Environmental Microbiology, 2007. **73**(6): p. 1712-1720.
- [2] Panacek, A., L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V.K. Sharma, T. Nevecna, and R. Zboril, *Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity*. Journal of Physical Chemistry B, 2006. **110**(33): p. 16248-16253.
- [3] Navarro, E., F. Piccapietra, B. Wagner, F. Marconi, R. Kaegi, N. Odzak, L. Sigg, and R. Behra, *Toxicity of Silver Nanoparticles to Chlamydomonas reinhardtii*. Environmental Science & Technology, 2008. **42**(23): p. 8959-8964.
- [4] Navarro, E., A. Baun, R. Behra, N.B. Hartmann, J. Filser, A.J. Miao, A. Quigg, P.H. Santschi, and L. Sigg, *Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi*. Ecotoxicology, 2008. **17**(5): p. 372-386.
- [5] Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, and M.H. Cho, *Antimicrobial effects of silver nanoparticles*. Nanomedicine-Nanotechnology Biology and Medicine, 2007. **3**(1): p. 95-101.