Cysteine can alleviate AgNP-induced oxidative stress

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Introduction

Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials in various fields of industry. Wide use of AgNPs in a broad range of commercial products has led to their increased release into the environment.[1] They have already been detected in water and soil, and will inevitably be taken up by crops and enter into the food chain, posing a risk for human health.[2] In this study we have analysed the effects of AgNPs stabilized with polyvinylpyrrolidone (PVP) on oxidative stress response in tobacco seedlings (*Nicotiana tabacum* L.). To examine if AgNP toxicity is NP-specific or it derives from dissolved Ag^+, cysteine, a ligand with a strong affinity for Ag, has been applied.

Materials and methods

Two weeks old tobacco (*Nicotiana tabacum* L.) seedlings were treated with 25, 50 and 100 µM of AgNP-PVP. To estimate the contribution of dissolved Ag^+ to the effects of AgNPs, 125, 250 and 500 µM of cysteine has been applied. Size distribution and zeta potential of the AgNPs were measured using dynamic light scattering (DLS, Malvern, UK). Silver content in the plant tissue was determined using inductively coupled plasma mass spectrometry (ICP-MS)[3]. Determination of the ROS level was performed using a dihydroethidium (DHE) test.[3] Activities and changes in isoform profiles of enzymatic antioxidants superoxide dismutase (SOD)[4], catalase (CAT)[5] and pyrogallol peroxidase (PPX)[6] were measured spectrophotometrically and by staining for their activity using native protein polyacrylamide gel electrophoresis (PAGE).

Results

Table 1. Physico-chemical characteristics of AgNP-PVP in ultrapure water by means of hydrodynamic diameter (d_H) in nm obtained from size distribution by volume, ζ potential values (mV) and SPR peak (nm).

Characteristics		AgNP-PVP
Size peak I	d_H, nm	8.16 ± 0.37
	Mean volume, %	81.28 ± 39.96
Size peak II	d_H, nm	41.53 ± 1.14
	Mean volume, %	1.05 ± 0.96
ζ potential, mV		-1.0 ± 0.6
SPR peak, nm		420

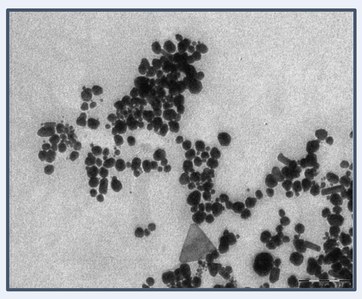
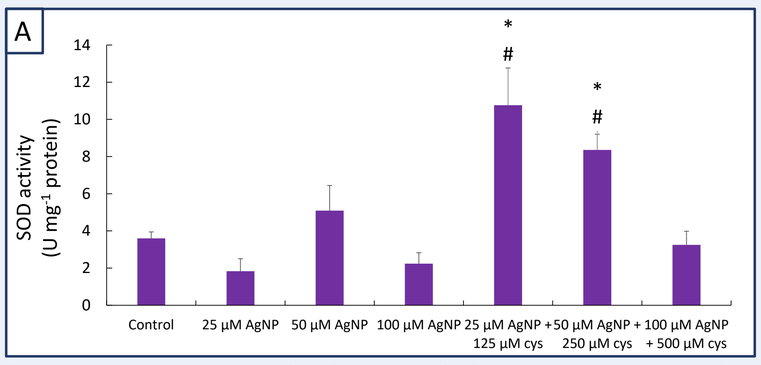


Figure 1. TEM image of AgNP-PVP.



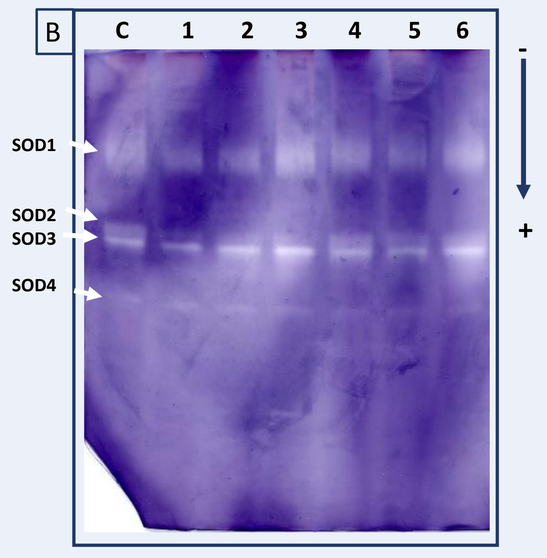
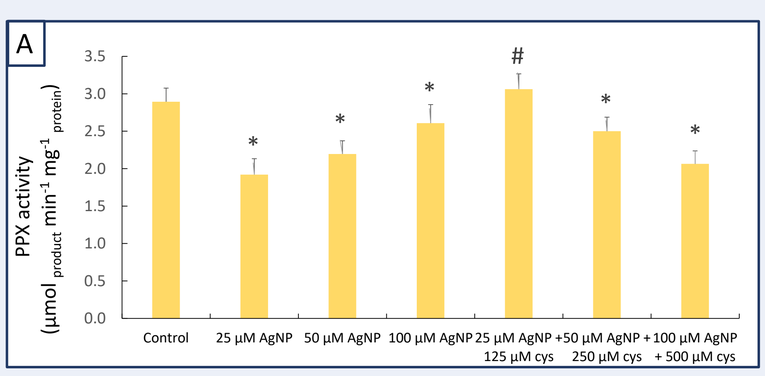


Figure 3. SOD activity (A) and isoforms in gel after native-PAGE (B) in tobacco seedlings treated with AgNP-PVP, alone or in combination with cysteine. Values are means ± SE of two different experiments, each with six replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine. C – control, 1 – 25 µM AgNP, 2 – 50 µM AgNP, 3 – 100 µM AgNP, 4 – 25 µM AgNP + 125 µM cys, 5 – 50 µM AgNP + 250 µM cys, 6 – 100 µM AgNP + 500 µM cys

Table 2. Silver content in tobacco seedlings treated with AgNP-PVP, alone and in combination with cysteine. Values are means ± SE of three replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine.

treatment	concentration	silver content
control	0	0
AgNP	25 µM	39.57 ± 5.79*
	50 µM	42.92 ± 3.85*
	100 µM	45.30 ± 4.29*
AgNP + cysteine	25 µM + 125 µM	14.34 ± 1.49*#
	50 µM + 250 µM	22.72 ± 0.89*#
	100 µM + 500 µM	21.14 ± 3.14*#



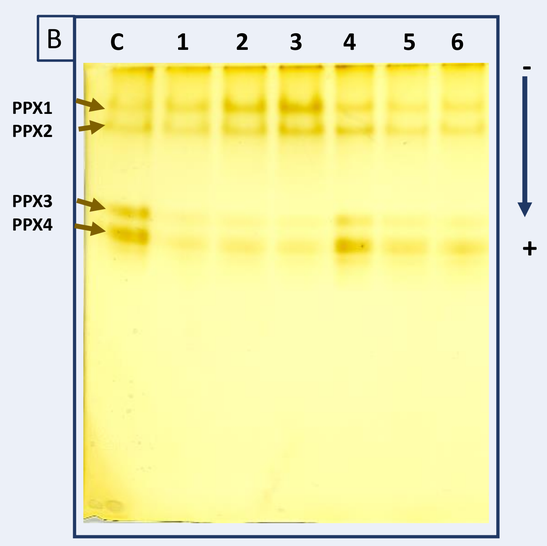


Figure 4. PPX activity (A) and isoforms in gel after native-PAGE (B) in tobacco seedlings treated with AgNP-PVP, alone or in combination with cysteine. Values are means ± SE of two different experiments, each with six replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine. C – control, 1 – 25 µM AgNP, 2 – 50 µM AgNP, 3 – 100 µM AgNP, 4 – 25 µM AgNP + 125 µM cys, 5 – 50 µM AgNP + 250 µM cys, 6 – 100 µM AgNP + 500 µM cys

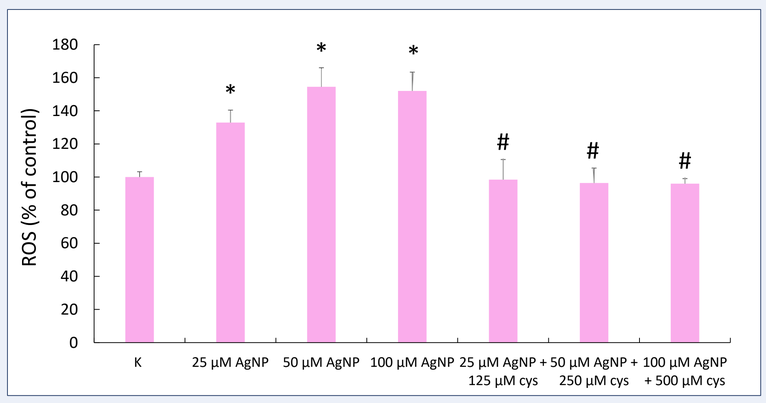
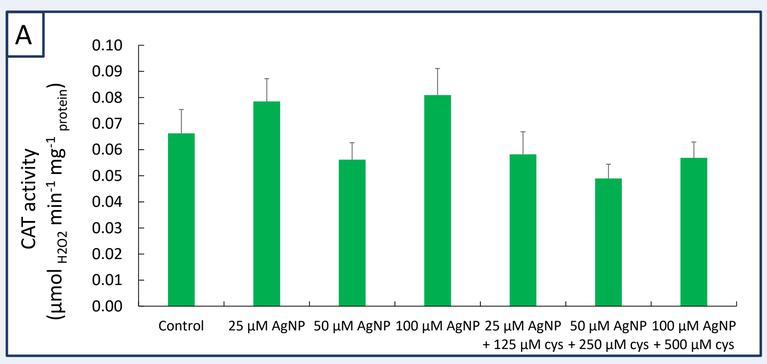


Figure 2. ROS content in tobacco seedlings treated with AgNP-PVP, alone and in combination with cysteine. Values are means ± SE of two different experiments, each with six replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine.



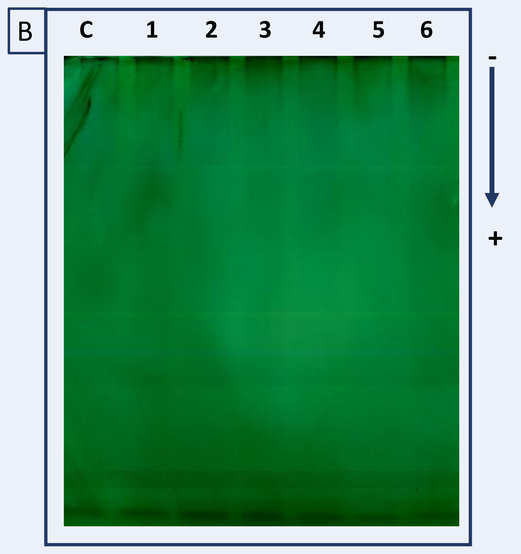


Figure 5. CAT activity (A) and isoforms in gel after native-PAGE (B) in tobacco seedlings treated with AgNP-PVP, alone or in combination with cysteine. Values are means ± SE of two different experiments, each with six replicas. C – control, 1 – 25 µM AgNP, 2 – 50 µM AgNP, 3 – 100 µM AgNP, 4 – 25 µM AgNP + 125 µM cys, 5 – 50 µM AgNP + 250 µM cys, 6 – 100 µM AgNP + 500 µM cys

Conclusion

- Significant and dose dependent increase of Ag content and ROS level in the plant tissue treated with AgNP-PVP decreased when cysteine was applied.
- No significant change in SOD activity was measured in AgNP-PVP treated seedlings, but addition of cysteine significantly enhanced SOD activity. Isoenzyme patterns revealed a difference in expression of certain isoforms in AgNP-treated seedlings compared to the control and combined AgNP-cysteine treatment.
- PPX activity significantly decreased in AgNP-treated seedlings, which was mitigated with the addition of cysteine only in the lowest applied concentration (25 µM). The same effect was shown by monitoring changes in expression of PPX isoforms.
- CAT activity did not change in either treatment, and no bands were detected on the gel.
- AgNP-PVP can cause oxidative stress in tobacco seedlings, and their phytotoxic effect at least partially derives from dissolved Ag^+.

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Literature

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