Proceedings of the World Congress on Recent Advances in Nanotechnology (RAN'16)
Prague, Czech Republic - April 1 - 2, 2016
Paper No. ICNNFC 114
DOI: 10.11159/icnnfc16.114

Proteomic Study of the Outer Layer of Biogenic Selenium Nanoparticles

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Extended Abstract

Selenium nanoparticles (SeNPs), which range in size from 50 to 300 nm, show broad applications such as antioxidant, immunoprotective, anti-tumor, antimicrobial and antobiofilm activities [1]. They also can be exploited in bioremediation, production of quantum dots and biosensors.

SeNPs can be synthesized chemically (ChSeNPs) or biologically (BioSeNPs). BioSeNPs can be obtained by various microorganisms, plant extracts or enzymatic preparations. Biosynthesis offers advantages over chemical synthesis such as minor costs, absence of toxic by products and energy saving (i.e. carried out under mild environmental conditions). BioSeNPs also present an outer layer, mainly consisting of a proteinaceous material, that seems to greatly influence the reactivity of SeNPs in terms of antimicrobial and antibiofilm effects. It has been proved that antimicrobial activity of BioSeNPs is significantly more than ChSeNPs.

To better understand possible mechanisms of this antimicrobial/antibiofilm efficacy of BioSeNPs, the nature of their outer layer should be characterized in details. In fact, the description of such surface-associated proteins and specificity of their binding to metal nanoparticles might allow to formulate new hypotheses on the biosynthetic route of SeNPs. Likely, nanoparticle-associated proteins are involved in the synthesis and maturation of SeNPs themselves.

This study focuses on the proteomic characterization of BioSeNPs external layer. For biogenic production of SeNPs, we used *Bacillus mycoides* SeITE01, an environmental strain isolated from the selenium-hyperaccumulator legume *Astragalus bisulcatus* rizosphere, capable of tolerating up to 25mM selenite [2].

The first step of the research was the characterization of protein associated to BioSeNPs through SDS-PAGE and mass spectrometry after 24 hours growth in the presence of selenite. Moreover, we studied the specificity of the protein-NP bond. Since ChSeNPs can also bind proteins when exposed to a cell free protein extract, a comparison between proteins associated to BioSeNPs and exposed ChSeNPs is currently ongoing.

We identified BioSeNPs-associated proteins for *B. mycoides*, which belong to primary and secondary metabolism, especially protein and amino acid metabolisms. As expected, proteins capable of reductase activity were found which are possibly involved in selenite reduction to zero-valent SeNPs: pyridine-nucleotide disulphide oxidoreductase, enoyl-ACP reductase (fatty acid biosynthesis) and FMN-dependent NADH azoreductase. Some membrane transporter and proteins involved in cell wall metabolism were also found, such as: penicillin-binding protein and lysozyme (peptidoglycan synthesis and degradation pathways, respectively) and ABC transporters. Several proteins involved in polypeptide synthesis and aminoacid metabolism were identified including: elongation factors Tu and G, ribosomal proteins, peptidases and a protease; glutamate and alanine dehydrogenases. Some of these are also involved in the sporulation process: elongation factors, ATPase, glyceraldehyde-3-phosphate dehydrogenase, enoyl-ACP reductase, a tellurium resistance protein and azoreductase. Actually, the sporulation mechanism might be used by bacterial cells to export nascent nanoparticles outside the cell. Finally, some of the proteins associated to BioSeNPs were found on exposed ChSeNPs as well.

In conclusion, we identified proteins that are most probably dealing with synthesis or maturation of the SeNPs, only surrounding BioSeNPs. This study can open the way to interesting applications for BioSeNPs, especially as an antimicrobial agent or in drug delivery systems.

References

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