Routes of internalisation and subcellular distribution patterns of metal-bearing nanoparticles in mussel, *Mytilus galloprovincialis,* as a function of nanoparticle characteristics.

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The aim of the present investigation is to contribute to the understanding of internalisation and subcellular distribution of metal-bearing nanoparticles (NPs) in different cell compartments within the digestive gland of the marine mussel *Mytilus galloprovincialis* depending on NP physico-chemical characteristics (size, additives, solubility, dispersion). At the subcellular level, transmission electron microscopy (TEM) and X-ray microanalysis were applied after *in vivo* short-term exposure (3 d) of mussels to different concentrations of NPs (TiO_2, ZnO, Au, Ag andCdS QDs -quantum dots-) of different sizes either with or without additives such as citrate, maltose or the surfactant DSLS. Small NPs (5-20 nm*:* Au5-Cit; Ag20-Mal) produced small aggregates (15-25 nm) and mid-sized aggregates (25-40 nm) that were readily incorporated *via* phagocytosis and endocytosis, respectively, into the digestive cell endo-lysosomal system. The internalisation of large NPs (>40 nm: Au40-Cit; Ag40-Mal; Ag90-Mal) in digestive cell lysosomes was less marked, most likely because only individual NPs followed the endocytic route. After exposure to TiO_2, Au and Ag NPs and CdS QDs, electron-dense particles were observed in the lumen of the digestive diverticula, associated to cell debris (e.g., within heterolysosomes and residual bodies). Whilst TiO_2 and Au NPs and CdS QDs remained apparently unchanged after being processed within the acidic endo-lysosomal system of the digestive cells, the size of Ag NPs was reduced in the heterolysosomes, which has been interpreted as the result of a partial dissolution of internalised NPs. Unlike in the former cases, electron-dense particles resembling NPs were not clearly observed in the case of ZnO NP exposures, except some scant electron-dense particles that were only occasionally found in the lumen of the digestive gland diverticula and the stomach, and in the blood sinuses. It seems plausible that ZnO NPs are rapidly dissolved. As a whole, the subcellular distribution of metal-bearing NPs is seemingly dependent on their size and solubility (this latter being affected by the presence of additives); which determine the uptake mechanisms in digestive cells (e.g. endocytosis), their fate in the endo-lysosomal system and the mode of release from digestive cells.

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