Transformations of Biologically Conjugated CdSe Quantum Dots Released into Water and Biofilms

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Abstract

Regarding CdSe quantum dots in soil and water, where bacterial interactions will occur, this project is addressing the following questions:

- What are the biotic fates of CdSe QDs?
- What are the effects of QDs on bacteria?
- How do fates and effects depend on: QD conjugation? QD size? Environmental factors (e.g., light, pH, reduction potential, oxygenation)? Bacterial strain? Growth habit (biofilm or planktonic)?

Thus far, the results have facilitated a working conceptual model of strain-dependent quantum dot interactions with bacteria:

- External nonspecific labeling where unconjugated QDs cannot passively diffuse into cells[1,2].
- Specific binding and nonspecific uptake[2] where conjugates are recognized by receptors on the cell envelope, and light-mediated membrane damage from oxygen free radicals facilitates transmembrane transport; thus allowing conjugated QDs less than 5 nm diameter to enter cells.
- External breakdown and transmembrane diffusion of constituent metals, where Cd^{2+} may be expelled; Se^{2-} may be expelled or oxidized to Se^{0} and then retained or expelled; or Cd^{2+} and Se^{2-} may combine into nanoparticles and be expelled or retained.

Specific results in support of this working model were generated from studies involving several bacterial strains. In *Bacillus subtilis*, conjugated quantum dots produced in the dark but incubated in visible light with bacteria results in the brightest bacterial labeling and the least amount of blue shift. This result supports the concept that CdSe quantum dots, when exposed to light in water, produce oxygen free radicals that impart membrane damage, allowing conjugated quantum dots to enter cells[2]. However, light induced membrane damage, as shown by transmission electron microscopy (TEM), appears to be transient because after a short time most cells are indicated in epifluorescence microscopy by a stain specific for viability. In *Escherichia coli*, conjugated quantum dots are taken in by similar mechanisms, but are then expelled after an hour.

Genotoxicity apparently occurs in *B. subtilis*, as evidenced by cellular elongation or the absence of cell division. Elongated cells are observed to contain CdSe rich occlusions, and several nuclear regions.

Toxicity of constituent metals is observed in *Pseudomonas* spp. liquid culture, whereby rapid growth occurs with selenite and slower growth occurs with Cd2+, whether alone or with selenite. In that growth with CdSe quantum dots is somewhere between growth without Cd2+ and with Cd2+, it is likely that some quantum dots are breaking down and causing toxicity, while some are intact outside the cells. Under aerobic conditions, reduction of selenite to selenide and elemental selenium is occurring, and this may occur intracellularly as well.

Toxicity of Cd2+ to planktonic *Pseudomonas* is exacerbated by 200 mg/L selenite, and the production of fluorescence for this condition suggests that nanoparticles might be forming intracellularly, or at least very near the cells. In any case, whether quantum dots are entering the cell whole or breaking down and then entering, the co-occurrence of Cd2+ and large amounts of Se2-, which is facilitated by the presence of quantum dots, appears to be more toxic than either metal alone.

When cultivated as unsaturated biofilms, the total yield of DNA in *Pseudomonas* is not affected by equimolar amounts of metals either in the form of quantum dots or as constituent metals. Cadmium uptake is greater for the constituent metal than for quantum dots. Lastly, cadmium appears to enhance intracellular accumulation of selenium, which may imply intracellular reduction of selenite followed by Cd2+ binding inside the cell.

In conclusion, work to date includes: (1) a putative, conceptual model for how quantum dots interact with bacteria; (2) evidence to support several interaction mechanisms, including light-activated QD production of membrane-damaging free radicals, free radical-assisted transmembrane transport of conjugated QDs, and conjugated QD-mediated DNA damage, arresting cellular division; and (3) early evidence for intracellular quantum dot assembly in biofilms (Cd-enhanced intracellular sequestration of Se) and in planktonic culture (fluorescence and exacerbation of Cd2+ toxicity when high amounts of selenite are present).

References

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