Charge-associated effects of fullerene derivatives on microbial structure integrity and central metabolism

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ESPP2 Environmental Stress Pathway Project

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Abstract

The effects of four types of fullerene compounds (C_{60}, C_{60}-OH, C_{60}-COOH, C_{60}-NH_2) were examined on two model microorganisms (*Escherichia coli* W3110 and *Shewanella oneidensis* MR-1). Positively charged C_{60}-NH_2 at concentrations as low as 10 mg/L inhibited growth and reduced substrate uptake for both microorganisms. Scanning Electron Microscopy (SEM) revealed damage to cellular structures. Neutrally-charged C_{60} and C_{60}-OH had mild negative effects on *S. oneidensis* MR-1, whereas the negatively-charged C_{60}-COOH did not affect either microorganism's growth. The effect of fullerene compounds on global metabolism was further investigated using [3-^{13}C]L–lactate isotopic labeling, which tracks perturbations to metabolic reaction rates in bacteria by examining the change in the isotopic labeling pattern in the resulting metabolites. The ^{13}C isotopomer analysis from all fullerene-exposed cultures revealed no significant differences in isotopomer distributions from unstressed cells. This result indicates that microbial central metabolism is robust to environmental stress inflicted by fullerene nanoparticles. In addition, although C_{60}-NH_2 compounds caused mechanical stress on the cell wall or membrane, both *S. oneidensis* MR-1 and *E. coli* W3110 can efficiently alleviate such stress by cell aggregation and precipitation of the toxic nanoparticles. The results presented here hypotheses that fullerenes cause more membrane stress than perturbation to energy metabolism.

Methods and materials

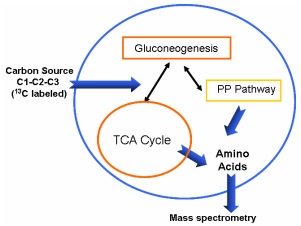

Figure 1.Schematics for the isotopic approach of investigating cellular global metabolism via ^{13}C labeling pattern in amino acids. Labeling patterns of metabolites were used to evaluate the perturbation of central carbon metabolism in this study.

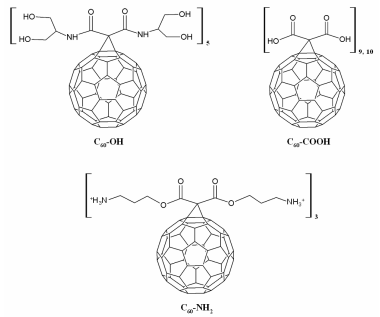

Figure 2. C_{60} fullerene derivatives used in this study. The fullerene-COOH-derivative carries negative charges in solution, the fullerene-NH_3^+-derivative is positively charged. The C_{60}-serinol (-OH) is neutral.

Experimental Results and discussions

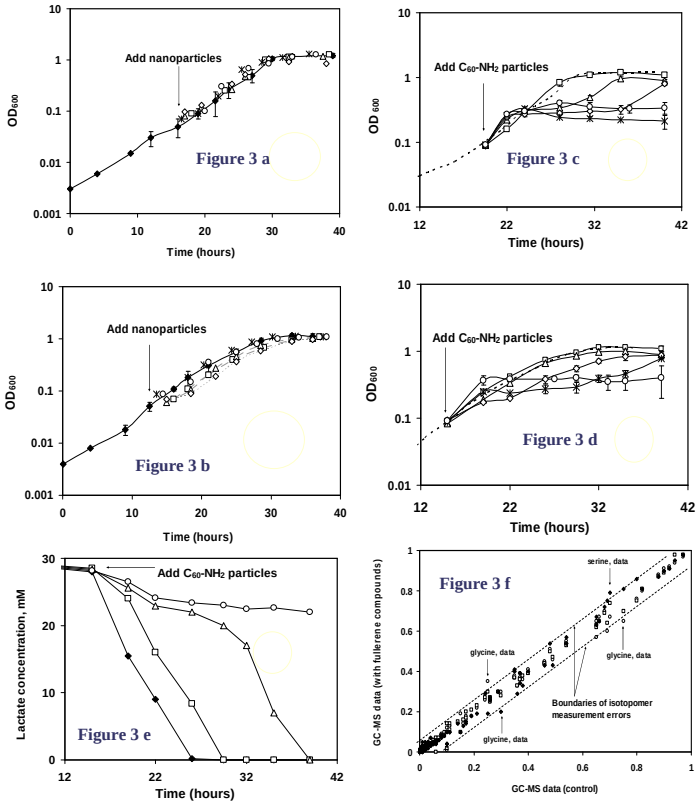

Figure 3. The effect of C_{60}, C_{60}-OH, C_{60}-COOH fullerene compounds (neutral or anionic charge) on *E. coli* W3110 **(Figure 3 a)** and *S. oneidensis* MR-1 **(Figure 3 b)** growth. ♦, control, 0 mg/l; □, C_{60}, 20 mg/l; Δ, C_{60}-OH, 20 mg/l; ◊, C_{60}-OH, 80 mg/l; *, C_{60}-COOH, 20 mg/l; ○, C_{60}-COOH, 80 mg/l; C_{60}-NH_2 fullerene (cationic charge) affected *E. coli* W3110 growth **(Figure 3c)** and *S. oneidensis* MR-1 growth **(3d),** and *S. oneidensis* MR-1 lactate uptake **(Figure 3e).** 0 mg/l; □, 1 mg/l; Δ, 10 mg/l; ◊, 20 mg/l; *, 40 mg/l; ○, 80 mg/l. (c) ♦, 0 mg/l; □, 20 mg/l; Δ, 40 mg/l; ○, 80 mg/l. Isotopomer distribution in proteogenic amino acids of *S. oneidensis* MR-1 cultured in [3-^{13}C] L-lactate medium **(Figure 3f).** The GC-MS data include 14 amino acids ((M57)+ and (M159)+ mass values) for all three nanoparticle-stressed experiments (○ 80 mg/L C_{60}-OH; □ 80 mg/L C_{60}-COOH; ♦ 20 mg/L C_{60}-NH_2).

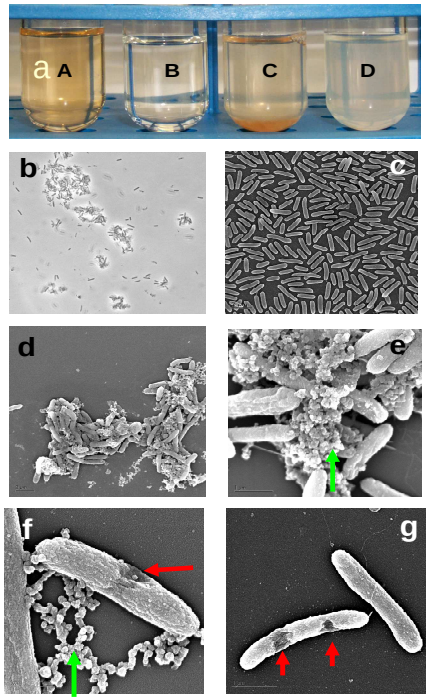

Figure 5. Images of *S. oneidensis* MR-1 exposed to C_{60}-NH_2. (a) Precipitation of *S. oneidensis* MR-1 with nanoparticles (A: no cells, C_{60}-NH_2; B: no cells, no nanoparticles; C: MR-1 cells at OD_{600} 0.26, C_{60}-NH_2 added; D: MR-1 at OD_{600} 0.26, no nanoparticles). (b) Light microscopy showed cell aggregation. (c) SEM of *S. oneidensis* MR-1 (no effect from NPs). (d) SEM of MR-1 aggregation in the presence of C_{60}-NH_2. (e) SEM of MR-1 in the presence of C_{60}-NH_2 (green arrow points to nanoparticles). (f) SEM of MR-1 in the presence of C_{60}-NH_2 aggregation (red arrow points to the damaged part of the cell). (g) SEM of individual MR-1 cells (red arrow points to the damaged part of the cell).

Conclusion

1. **Positive charged nanoparticles are more toxic.**
2. **Central metabolism is not altered by presence of nanoparticles.**
3. **Bacteria are able to remediate toxic nanoparticles.**

ACKNOWLEDGEMENT

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