Microbial Cell Factories

Poster Presentations

Cellular toxicity triggered by bacterial inclusion bodies

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Background

The cytotoxicity associated with inclusion bodies and its possible impairment to recombinant protein production processes in *E. coli*, have been poorly studied so far [1]. To explore these possible toxicity mechanisms we have employed a well-described system used with other kinds of protein aggregates to evaluate deleterious effects in animal cells. Cytotoxicity in animals has been associated not only with a range of proteinaceous aggregates in several degenerative diseases but also with prefibrilar aggregates of protein unrelated to any clinical diseases since they have been reported to harm cell viability by affecting biochemical parameters such as intracellular redox status and free Ca^{2+} levels [2]. In this context this work contributes to support the hypothesis that misfolded proteins cause negative cellular effects as a result of exposition of hydrophobic patches or other aggregate characteristic structures on its surface.

Results

In this study we analysed the cellular toxicity associated with inclusion bodies formed in MC4100 *E. coli*, producing the misfolding-prone polypeptide VP1LAC, consisting on a N-terminal β-galactosidase fusion containing the VP1 capsid protein of foot-and-mouth disease virus [3]. The protein aggregates produced during either 1 h or 5 h were added at a range of final protein concentrations (from 1 μM to 8.5 μM) to NHI-3T3 cells. We demonstrated by confocal microscopy analysis that bacterial inclusion bodies bind and enter into the cells. Moreover its cytotoxic effect was evaluated by MTT reduction assay, a standard indicator of cell physiological state. As shown in figure 1, the experiments reveal that IB significantly impairs cell viability mainly when were incubated with 5 h-inclusion bodies. Interestingly the thermal aggregates of β-galactosidase obtained *in vitro* by a temperature shift to 96°C and further incubation at room temperature, do not significantly modify MTT reduction in comparison to control wells or to cells incubated with soluble β-galactosidase.

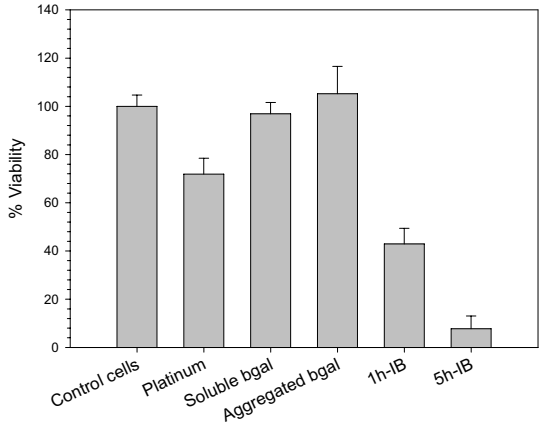

Figure 1
Percentage of cell viability respect to control cells in NIH-3T3 cultures incubated for 24 hours with 4 μM of 1 h- or 5 h-aged inclusion bodies, soluble β-gal and thermal aggregated β-gal. Cells incubated with platinum are included in the experiment as a citotoxicity positive control.

Conclusion

In the present study, we prove that bacterial inclusion bodies are clearly toxic for mammalian cells. Despite the fact that it has been previously suggested that the most highly cytotoxic aggregates are the early prefibrilar assemblies rather than mature fibrils [2], in our case the more structured 5 h-aged inclusion bodies show a more pronounced toxic effect compared to those formed only during 1 h. However, the β-gal thermal aggregates, which have been shown to present a fibrilar pattern, behave as expected since they have not been associated with any extent of toxicity. Overall, these results support the hypothesis that different kinds of aggregates are deleterious but encourage us to study the rules that regulate the proteinaceous aggregates' toxicity.

References

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