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Poster Presentation

Models for the study of inclusion bodies formation as a function of fermentation conditions and protein sequence

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Background

The building of aggregates of variable complexity is often observed in bacterial host cells upon over-expression of recombinant proteins. This event is thoroughly studied as it impacts on the production of recombinant proteins and also as a model to investigate the molecular and physiological factors producing protein aggregation in living cells [1]. In *E. coli*, a network of molecular chaperones assists protein folding and re-folding [2]. Recent experiments showed that aggregation reversion can be improved as protein synthesis is interrupted and that the ratio protein/chaperons as well as the kind of chaperons enclosed in inclusion bodies varies according to the physiology of overproduction [1,3,4].

Stability, solubility and propensity of a protein to aggregate both in inclusion bodies and in amyloid structures have been related to its polypeptide sequence [5]. This study aims at gaining a deeper insight in the composition and kinetics of aggregate formation and to relate this information to the molecular features of the recombinant expressed proteins.

Results

We have studied the behaviour of three different proteins over-expressed in *E. coli* by commercial expression vectors regulated by IPTG. The three model polypeptides display different features: i) the cold active lipase from *Pseudomonas fragi* is very unstable even at moderate temperature and, therefore, is a very sensitive tool to investigate the temperature-dependent aggregation development [6]; ii) the green fluorescent protein-glutatione S- transferase fusion protein enables to monitor residual fluorescence in aggregated proteins and, as a consequence, to evaluate the extent of residual native structure; iii) wild type and mutated lactoglobulins have been used as a probe to test the effect of changes in the amino acid sequence on the protein stability during recombinant expression. Ratio of soluble to insoluble proteins has been evaluated by SDS PAGE and activity has been measured in both fractions, whenever applicable. Additional information has been provided by structural analysis performed by Fourier transform infrared spectroscopy. In all the models the fermentation temperature has been identified as a major determinant of the total amount and rate of aggregation as well as of the complexity, compactness and residual native- like structure of inclusion bodies [4-7]. Moreover, concentration of DnaK inside inclusion bodies has been followed by Western-blot analysis and a correlation with the amount of insoluble protein has been detected.

Conclusion

Kinetics of aggregation, content and residual protein structure and activity in *E. coli* inclusion bodies are features highly protein specific but also dependent on the conditions under which aggregation occurred. This last observation suggests that the precise monitoring of recombinant protein aggregation during the fermentation

can lead to a system in which the optimisation of the growth conditions is automatically set with the biotechnologically relevant increase of soluble protein yields. Furthermore, the monitoring of the aggregation dynamics specific for the different mutants might provide valuable indications to engineer species with higher solubility.

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