Microbial Cell Factories

BioMed Central

Oral Presentation

Investigation of the inclusion body formation process by FTIR spectroscopy

Gerd Margreiter*[1], Manfred Schwanninger[2], Christian Obinger[2] and Karl Bayer[1]

Address: [1]Department of Biotechnology, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria and [2]Department of Chemistry, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria

Email: Gerd Margreiter* - gerd.margreiter@boku.ac.at

* Corresponding author

from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):S11 doi:10.1186/1475-2859-5-S1-S11

Background

The great demand on high amounts of pure protein for pharmaceutical applications and for research made *Eschericha coli* one of the most important cell factories for recombinant protein production. Although it is a well studied organism showing high productivity, the recombinant protein frequently aggregates and forms the so called inclusion bodies (IBs). The entire aggregation process is still poorly understood. Recent research showed that proteins expressed as inclusion bodies have extensive native-like secondary structures and that the formation of IBs is a result of specific aggregation between folding intermediates of protein molecules. However, solubilisation of inclusion bodies by application of high concentrations of chaotropic reagents the secondary structure is destroyed, leading to a random coil formation of the protein structure and exposure of hydrophobic surfaces. The loss of secondary structure due to solubilisation and the interaction of thereby exposed protein domains lead to undesired aggregation and misfolding of the target protein. These reactions are considered to be responsible for poor recovery of bioactive proteins from IBs [1-4]. Therefore, solubilisation processes which provide the conservation of the existing secondary structure of the IBs will probably lead to higher yields of bioactive protein. Consequently it is important to evaluate to which extent the structural properties of the IBs can be influenced by cultivation conditions and induction strategy [5].

For structural analysis the method of choice is FTIR (Fourier Transformed Infrared) spectroscopy, because of its possible application to study proteins regardless their physical form both in solution and in solid precipitates. FTIR is very sensitive for analysis of the secondary structure and for detecting conformational changes.

Results

Solubilisation of IBs with different buffers and refolding

To find an alternative solubilisation method, a test series with buffers of different pH and urea concentrations was performed. Using Tris/Cl pH11.4 containing 3–5 M urea, as well as Tris/Cl pH11 containing 4 and 5 M urea the IBs of the model protein (autoprotease-GFP fusion protein) were dissolved completely (also with 8 M urea pH8 as a standard method). Refolding was performed by dilution and showed a higher yield for IBs which were dissolved by buffer with high pH and less urea compared to IBs dissolved by 8 M urea pH8. FTIR spectroscopy of the protein dissolved in the different buffers (8 M urea vs. 4 M urea pH11.4) showed shifts of the amide I band, indicating differences of the secondary structure.

Impact of cultivation conditions on IB structure:

Harvested inclusion bodies of four fed-batch cultivations differing in cultivation temperature (30°C and 37°C) and induction strategy (partial and full induction with IPTG) have been analysed by ATR-FTIR-spectroscopy. IBs of the

cultivation at 30°C and full induction showed a shift of 2 wavenumbers compared to the others, what could be an evidence for differences in the structure, and the refolding experiment resulted in a higher yield. Furthermore different ratios of soluble and insoluble recombinant protein fractions were obtained at the different cultivation conditions.

Conclusion

Experiments with alternative dissolving conditions compared to the standard method (complete unfolding with high concentrations of chaotrops) demonstrated the high potential of conserving existing protein structures in inclusion bodies to obtain higher refolding yields. In addition, data obtained by FTIR spectroscopy and the different ratios of soluble to aggregated fraction confirm the impact of different cultivation conditions on the folding process of the heterologous expressed proteins. Furthermore, the recent observations of the heterogeneity of folding states in inclusion bodies points out the demand of a new view of those aggregates. Monitoring the entire folding process of the recombinant protein combined with improved downstream strategies has a high potential to increase renaturation efficiency and to improve protein quality. Further research on diverse proteins is necessary to get deeper insight into inclusion body formation.

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