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Poster Presentation

Modulation of inclusion body (IB) formation kinetics by different induction regimes in *E. coli* fed batch cultivations

Monika Cserjan-Puschmann*, Franz Clementschitsch, Gerald Striedner, Florentiner Pötschacher, Jürgen Kern and Karl Bayer

Address: Department of Biotechnology, Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences, Vienna, Austria

* Corresponding author

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Background

Heterologous protein expression often results in the accumulation of non-native insoluble, quite homogenous protein deposits inside bacterial cells, called inclusion bodies (IBs) [1,2]. Currently there is a strong trend to favour IB formation in industrial recombinant protein production processes, because IBs are a source of relatively pure and stabilised proteins. Therefore, the major objectives of process optimisation are to achieve a controllable, stable and nearly complete IB formation and IBs of high quality.

IB formation is a complex multifactorial synthetic pathway (transcription, translation, folding, aggregation) that depends on the specific host/vector system, the intrinsic characteristics of the protein, the physiology of the host cell and the process operation (cultivation temperature, growth rate, media composition, etc). However, the precise mechanisms of the in-vivo protein aggregation process remain poorly understood. The balance between heterologous and host protein production rate plays a fundamental role in IB formation. Therefore, varying metabolic loads gained by different induction regimes are applied to control kinetics of IB formation.

Host/vector system: *E. coli* HMS174(DE3) pET30a producing a codon-optimised GFP fusion protein (47 kDa) showing strong tendency to form IBs. To investigate IB formation kinetics a series of exponential carbon-limited fed-batch cultivations were performed at different induction levels of recombinant protein expression. Transcription rate control was achieved by continuous supply of limiting amounts of the inducer (IPTG) in a constant ratio to biomass [3]. During cultivation yield of recombinant protein, composition of IBs, distribution ratio of soluble and aggregated proteins, IB quality in consideration of the subsequent downstream procedures, cellular growth (BDM), total cell number (TCN) were monitored. In addition the metabolic load on the host cells exerted by different inducer concentrations was quantified by the signal molecule ppGpp [4].

Results

In all cultivations of this model protein the impact of inducer level on the ratio of soluble and aggregated proteins was not significant, more than 96% of the total recombinant protein was deposited in IBs showing low concentrations of host cell proteins.

The key results of this series of fed batch cultivations demonstrated that the specific content of recombinant protein increases according to the inducer concentrations, whereby a higher yield of total biomass is obtained under low inducing conditions. The outcome of these experiments is that approximately the same total amount of recombinant protein could be produced whereby the IB formation rate showed strong differences.

In addition to the target protein (47 kDa) fragments of it with a length of about 20–35 kDa as confirmed by Western blots were observed. This inhomogeneity caused by incorrect protein translation account up to 40% of the expressed recombinant protein at the end of cultivation in particular at high induction levels. These results clearly demonstrate the benefial impact of lower metabolic burden on increased IB quality.

The varying induction levels differently affected the host cell system. During a non-induced cultivation the cells are exponentially growing and the ppGpp level is constant. Due to the synthesis of recombinant protein stringent response was triggered and in succession the cells lost their capability to divide in relation to the metabolic burden. IB formation kinetics can be classified into three phases: a growth associated product formation (phase I), a partial growth decoupled pase (II) without further cell division and a complete growth decoupled IB formation (phase III), where the recombinant protein is expressed at the expense of the cellular protein. However, cell viability could be maintained at low induction levels that cause a tolerable metabolic load.

Conclusion

The performed experiments showed close interrelationships between the level of induction, cellular growth, IB formation with respect to metabolic load. In summary it can be said that the inducer feed strategy that permits tuning of recombinant gene expression in relation to the metabolic potential of the host cell synthesis machinery is a valuable tool to attain maximal yield and quality of IBs. In future the alteration of the host cell physiology due to different induction regimes will be assayed using genomic and proteomic analysis.

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