Microbial Cell Factories

Poster Presentation

Use of the *tetA*-promoter in fed-batch cultivations: Repeated supply of anhydrotetracycline is necessary for production of tetrameric collagen prolyl 4-hydroxylase in *Escherichia coli*

A Neubauer*[1], J Soini[2], M Bollok[2], M Zenker[2], J Sandquist[2], J Myllyharju[1] and P Neubauer[2]

Address: [1]Dept. Medical Biochem. & Molec. Biology, P. O. Box 5000; Biocenter Oulu, University of Oulu, FIN-90014 Oulu, Finland and [2]Bioprocess Engineering Laboratory, Dept. Process & Environm. Engin., P. O. Box 4300, Biocenter Oulu, University of Oulu, FIN-90014 Oulu, Finland

* Corresponding author

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Background

Human collagen prolyl 4-hydroxylase (C-P4H), an ER lumenal protein, is a key enzyme in the biosynthesis of collagens and consists of two different subunits forming an $\alpha_2\beta_2$ tetramer. Heterologous cytoplasmic production of an active C-P4H in *Escherichia coli* using a bicistronic vector with the T5-*lac* and *tet* promoters and the strain Origami™ as a host was described earlier [1]. Gene optimisation of the β subunit that is identical to protein disulfide isomerase and selection of the best induction conditions improved the obtained activity of recombinant C-P4H in shake flask cultivations further by a factor of 50 [2].

Results

Although high amount of active C-P4H was obtained in shake flask cultures with long-time induction, the amount was low in fed-batch cultivations using the same induction strategy. Analysis of the mRNAs of the α and β subunits in fed-batch fermentations by Sandwich hybridisation revealed that single addition of the inducer anhydrotetracycline (aTc) leads to only transient induction of the α subunit, in contrast to IPTG which leads to a stable level of β mRNA.

Surprisingly, repeated induction with aTc led to a stable level of α mRNA and, consequently, to higher yields of the active C-P4H tetramers. The expression was strongly dependent on the cell density and the specific growth rate and provided best results if the culture was induced during the batch cultivation phase.

Conclusion

Our results indicate specific, so far non-described, features of the *tet* promoter based expression system. The repeated stepwise addition of aTc at higher cell densities might be of particular importance for other expression systems involving the *tet*A promoter and long expression time.

References

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