239h Kinetic Analysis of the Envelope Stress Response during the Temperature Induced Periplasmic Expression of Recombinant Streptokinase in Escherichia Coli

Balaji Balagurunathan and Guhan Jayaraman

Recombinant protein over-expression in Escherichia coli often elicits a stress response that leads to the up-regulation of stress genes and their products. The localization of the recombinant protein within the cell plays a crucial role in the response of the cell to stress. Studies on the mechanisms employed by Escherichia coli to sense and respond to stress have led to a greater understanding of protein processing within the cell. Separate cellular stress response systems serve each compartment in Escherichia coli, the cytoplasm and the envelope (which includes the inner membrane, periplasm and the outer membrane). The cytoplasmic heat shock response is controlled by the regulated proteolysis and chaperone-mediated inactivation of alternative sigma factor Sigma32 [1-3]. Escherichia coli senses and responds to extracytoplasmic stress via at least two overlapping, but distinct, transduction pathways, the Cpx two-component system and the heat shock sigmaE pathway. The Cpx pathway is induced by elevated pH, altered inner membrane composition and overproduction of envelope proteins or pili subunits. The sigmaE envelope stress response is induced by heat shock, ethanol shock or by perturbations to outer membrane protein folding [4].

A two plasmid system was used for the temperature induced secretory expression of recombinant streptokinase in Escherichia coli [6, 7]. T7 RNA polymerase was induced from a plasmid with a shift in temperature which in turn induces the production of the recombinant protein from the other plasmid (which carries the gene of interest under T7 promoter). Recombinant streptokinase was produced as an intracellular product (from the plasmid pRSETB-STK)/secretory product (from the plasmid pSSY4) and the expression levels were compared. The overall activity of recombinant streptokinase was 50 fold lower when produced as a secretory product (the size, copy number and antibiotic marker for the two plasmids being similar). It was also observed that the secretory production leads to enhanced proteolysis (as shown by the degradation bands). This regulated proteolysis of the pre-protein (protein with the signal peptide) could be the reasons for the lower overall productivity. From our experimental results we could conclude that degradation could be due to a membrane bound protease.

A detailed mathematical model was developed to explain the control architecture governing the stress response network in Escherichia coli. The stress response network basically involves the regulation of the alternative sigma factors sigma32 and sigmaE. The major cytoplasmic and periplasmic chaperones and proteases are a part of these regulon. The regulon function is essential during normal as well as stressful conditions. The disturbance to the stress response network due to strong over-expression of recombinant streptokinase by a temperature shift was analyzed using the model simulations. The simulations were compared with the experimental data on temperature induced recombinant streptokinase production. Some strategies to enhance productivities are explored with the model simulations.

References

1. Samad H. El., Kurata, H., Doyle, J. C., Gross, C.A. and Khammash, M., 2005. Surviving heat shock: Control strategies for robustness and performance. Proc. Nat. Acad. Sci. USA. 102: 2736-2741.

2. Arsene F, Tomoyasu T, Bukau B. 2000. The heat shock response of Escherichia coli. Int J Food Microbiol 55:3-9.

3. Srivastava R, Peterson MS, Bentley WE. 2001. Stochastic Kinetic analysis of the Escherichia coli stress circuit using sigma32-targeted antisense. Biotechnol Bioeng 75:120-129.

4. Raivio TL, Silhavy TJ. 2001. Periplasmic stress and ECF sigma factors. Annu Rev Microbiol 55:591-624.

5. Ades SE, Grigorova IL, Gross CA. 2003. Regulation of the alternative sigma factor sigmaE during Initiation, Adaptation, and shutoff of the extracytoplasmic heat shock response in Escherichia coli. J Bacteriol 185(8):2512-2519.

6. Yazdani S S and Mukherjee K J. (1998) Overexpression of streptokinase using a fed-batch strategy. Biotechnol. Lett. 20(10):923-927.

7. Yazdani S S and Mukherjee K J. (2002) Continuous-culture studies on the stability and expression of recombinant streptokinase in Escherichia coli. Bioprocess. Biosyst. Eng. 24:341-346.