Microbial Cell Factories

Poster Presentation

Production of recombinant mink growth hormone in *E. coli*

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Background

Growth hormones are produced by the anterior pituitary gland of vertebrates. Apart from stimulating linear body growth, it plays an important role in variety of metabolic and physiological processes. There is almost no information on mink growth hormone (mGH), which probably could be a useful factor in mink fur production, synthesis in *E.coli* and future recovery of bioactive protein. It is known that mGH gene was cloned and sequenced [1,2]. We report the possibility to produce large amounts of mGH using recombinant DNA technology.

Results

The host strain *E. coli* BL21(DE3) harbouring the plasmid pET21a+/mGH was grown in batch fermentation process at 37°C using nutrient rich medium to produce mGH. The target protein expression was induced with 0.2 mM or 1 mM of isopropyl-β-D-thiogalactoside at OD_{600} of 2.0. In both cases after 3 hours of induction the expression level was similar and equal to 23% and 27% of the total cellular protein, respectively. mGH when overexpressed in *E. coli* accumulated as inclusion bodies. After cell disruption by sonication inclusion bodies were purified by washing with water, then with cleaning solution (2 M urea, 1 M NaCl, 5 mM EDTA, 1 mM PMSF in 0.1 M Tris-HCl buffer pH 9.0) and once more with water. The washed inclusion bodies were found to contain approximately 80% of mGH. 8 M urea solution was used for its solubilization. mGH was refolded by dilution protocol in the presence of glutathione pair. The mGH conformational state was analyzed by RP-HPLC. Two-step purification process comprising of ion-exchange chromatography on Q-Sepharose and hydrophobic chromatography on Phenyl-Sepharose was developed. The biological activity of the purified mGH was assessed in vitro using a mouse myeloid cell line transfected with the full length ovine GH receptor [3]. Stimulation of cells was assessed using the MTT-formazan dye assay, that monitors both metabolic and mitogenic activity. 25–30 mg of highly purified and biologically active mGH was obtained from 4 g of biomass.

Conclusion

The method presented here allows producing large quantities of mGH and considering initiation of scientific investigations on mGH availability in fur industry.

References

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