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Expression and Purification of Recombinant Human Basic Fibroblast Growth Factor from Fed-Batch Cultivation of *E. coli*

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Introduction

Human basic fibroblast growth factor (hbFGF) is a single-chain, non glycosylated protein. It contains 155 amino acids, has a molecular weight of 17KDa and modulates both cell proliferation and cell differentiation in vitro and in vivo. As a mitogen, hbFGF is a potent mediator for wound healing, angiogenesis and tissue regeneration and has great potential in clinical treatment. In this study, *E. coli* BL21(DE3), transformed with a plasmid (pET-29c(+)),containing a recombinant human gene coding for hbFGF, was used for the production. Cultivations were carried out in a 2 L bioreactor using a defined medium as described by Anke S (Anke S, et al. Enzyme Microb. Technol.(1995) 17:947-953). In the feeding phase, the specific growth rate was controlled to 0.15 h^{-1} based on a process growth model in order to achieve large amounts of soluble proteins. After the cultivation, cell mass was collected and lysed by passing through a high pressure homogenizer for 6 times at 620 bar. Then, the supernatant was applied to a fast protein liquid chromatography (FPLC) system, by two step purification with strong cation exchange membrane and heparin affinity column respectively; homogeneity hbFGF with fully native conformation was obtained with an overall yield of 77%.

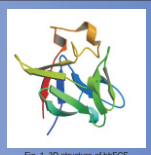

Fig. 1 3D structure of hbFGF (Zhu X,et al.Science.(1991) 251:90-93)

Overall process

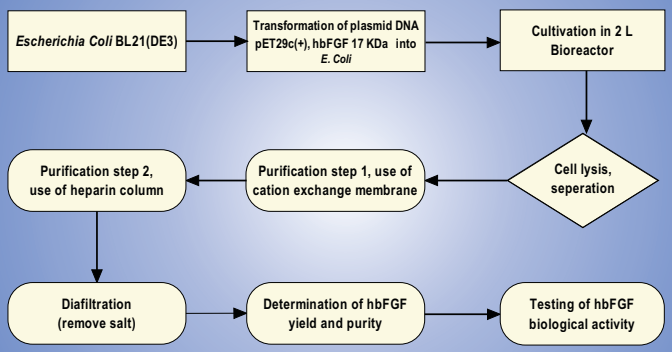

Fig. 2 Flow chart of hbFGF production from fed-batch cultivation of recombinant *E. coli*

Cultivation

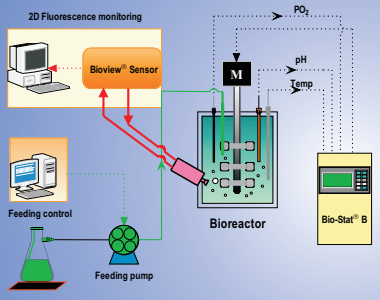

Fig. 3 Online process monitoring and control system of fed-batch cultivation. Dash line shows the signal flow. The final biomass was 18.9 g/L and hbFGF expressed was 20.5 mg/g dry cell weight with 88% soluble protein, as estimated by densitometry.

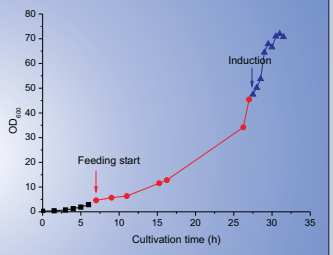

Fig. 4 Growth curve of *E. coli* in fed-batch cultivation. Feeding start at 7 h, with a controlled specific growth rate of 0.15 h^{-1},at 27.5 h protein expression was induced by adding 1mM IPTG.

Purification

Step 1: Cation exchange chromatography

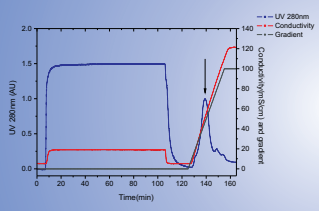

Fig. 5 Cation exchange chromatography of hbFGF. hbFGF was eluted by a linear gradient of 0-1M NaCl in 25 mM sodium phosphate buffer (pH 7.5) with a flow rate of 1 ml/min. hbFGF recovery rate was 80%.

Step 2: Heparin affinity chromatography

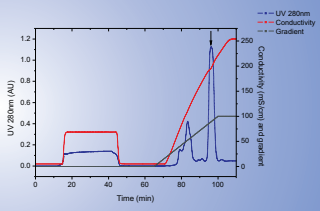

Fig. 6 Heparin affinity chromatography of hbFGF. hbFGF was eluted by a linear gradient of 0-2.5 M NaCl in 25 mM sodium phosphate buffer (pH 7.5) with a flow rate of 1 ml/min. hbFGF recovery rate was 77%.

Identification

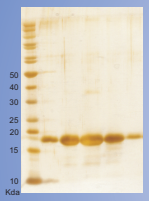

Fig.7 SDS-PAGE of two step purified hbFGF stained with silver. The single bands migrated between 15 and 20 KDa, which corresponded to hbFGF (17 KDa).

Fig. 8 Western blot of purified hbFGF on PVDF membrane. Using Monoclonal anti-human bFGF antibody (Sigma-Aldrich, Germany) as the primary antibody.

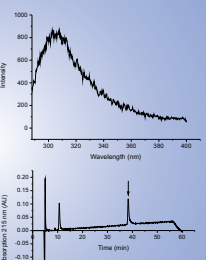

Fig. 9 Fluorescence spectrum of purified hbFGF. Using 280 nm as excitation wavelength. The asymmetric peak at 306 nm indicated that the protein was in complete native conformation.

Fig. 10 HPLC of purified hbFGF. Using a C4 column (4.6 x 250 mm, Macherey-Nagel, Germany). hbFGF eluted by a linear gradient of 0-50% acetonitrile containing 0.1% TFA, with a flow rate of 1 ml/min.

Activity test

To determine the biological activity of the purified hbFGF, NIH-3T3 cells were seeded into 6- well plates (in duplicates) and hbFGF was supplied to the culture medium the next day. To estimate the activity of our hbFGF we compared it to the activity of a commercial hbFGF from PeproTech Tebu-Bio. As a negative control, instead of any growth factor PBS was added to the culture medium. Cell numbers were determined daily by using a haemocytometer.

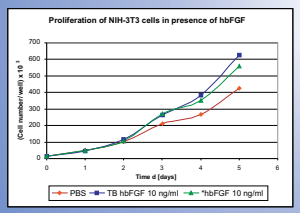

Fig. 11 Proliferation of NIH-3T3 cells in the presence of hbFGF.NIH-3T3 cells showed a higher proliferation rate when cultured in presence of hbFGF compared to the negative control (PBS). Our purified protein (*hbFGF) showed a similar activity on the cells as the commercial protein (TB hbFGF) according to the cell numbers after growth factor supplementation.

Summary

Using the *E. coli* expression system, hbFGF was produced in high yield. The combination of strong cation exchange chromatography and heparin affinity chromatography result in a very efficient protocol for purification of hbFGF produced with Fed-batch cultivation of *E. coli*. The produced hbFGF was tested successfully for biological activity using the fibroblast cell line NIH-3T3.

Compare to the existing expression and purification process, this process has advantages of high quantity expression, simple two-step downstreaming process, high protein purity and easy to scale up.

This project is granted by rebirth