HIGH LEVEL EXPRESSION AND SIMPLE PURIFICATION OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR IN E. coli

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SUMMARY

A human granulocyte colony-stimulating factor (hG-CSF) gene was synthesized and inserted into a trp expression vector for overexpression in E. coli. A strong expression vector was constructed, and a simple purification procedure including in vitro refolding was established. The final productivity of hG-CSF was 500~600 ng per l culture, and the purified hG-CSF showed the proliferation of neutrophils in vivo assays.

INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is one of a family of hematopoietic growth factors and regulates the proliferation and differentiation of cells of the neutrophil lineage (Zaebo et al., 1986). Recently, the cDNA encoding human G-CSF was cloned from the human bladder carcinoma cell line 5637 (Souza et al., 1986) and recombinant human G-CSF (rhG-CSF) is now available for clinical trials. Because the sequence bias of genes in nature and its correlation with tRNA are significantly different between procaryotes and eucaryotes, there is a limitation for the expression of human cDNA in E. coli system (Carrier et al., 1984). In fact, stable secondary structures in mRNA in the vicinity of the AUG codon are important obstacles for translation in procaryotes, presumably by blocking the binding or the movement of
ribosomes (Buell et al., 1985). Especially, the codon-anticodon interaction seems to be so sticky to interfere the translational initiation of hG-CSF in E. coli due to the abundance of GC rich codons in 5' end of hG-CSF cDNA (Grosjean and Fiers; 1962; Devlin et al., 1988). In this report, therefore, the strategy we used to increase the expression level was the use of entire synthetic gene and the improvement of mRNA stability. We also report the simple purification of rhG-CSF for the large-scale preparation using cation exchange and chromatofocusing chromatography.

**MATERIALS AND METHODS**

**Synthesis and cloning of hG-CSF gene**: A synthetic gene encoding the 175 amino acids of hG-CSF including the initiation codon was designed on the basis of the known amino acid sequence (Souza et al., 1986) for efficient expression in E. coli. Twenty-six oligonucleotides ranging in size from 38 to 46 mer were synthesized, purified, and assembled to form the synthetic hG-CSF gene. The synthetic hG-CSF gene was inserted into pUC18 via their EcoR1/HindIII ends. The resulting plasmid was designated as pCSF18 and used as gene source of G-CSF.

**Construction of expression vector**: Plasmid pCSF18 was digested with ClaI and SalI restriction enzymes, and a 540bp fragment containing the encoding region of hG-CSF was isolated. The DNA fragment was then subcloned between ClaI and SalI sites of plasmid pDA103. E. coli strain HB101 and DH5α were used as recipients for vector construction and E. coli strain W3110 (F−, hsd R−, hsd M+) was utilized as recipient of recombinant plasmid pCSF451.

**Fermentation**: The fermentation process was performed by fed-batch type. The batch medium consist of 30g/l bacto trypton, 5g/l yeast extract, 10g/l glucose, trace metals solution. A 25% casamomic acid solution which lacks tryptophan was used as a nitrogen source of feed medium in order to induce the production of hG-CSF effectively. 3-β-indoleacrylic acid (3-IAA) was added to 1mM at the early log phase of growth (A600=45), and incubated for an additional 6 hours. Cells were harvested by centrifugation and resuspended in 10mM Tris/HCl (pH 7.5), 1mM EDTA. The suspension of cells was disrupted by Manton-Gaulin homogenizer. Inclusion bodies were collected after centrifugation (12,000g, 30min) and dissolved in 2M urea together with pH shift to alkaline condition.

**Purification of hG-CSF**: The solution of inclusion bodies was diluted with 4 volumes of deionized H2O (pH 10.0) within 30 minutes after solubilization and renaturated spontaneously for 16 hours at room temperature. The renaturation process was terminated by pH shift to 5.5 with 1M H2PO4, and then a large amount of other proteins was precipitated. The supernatant was recovered by filtration and was loaded onto SP-Sepharose fast flow (Pharmacia, 10×50cm) equilibrated with 40mM sodium phosphate (Monobasic). The column was washed extensively with same buffer, and the bound proteins was eluted with 0~0.5M NaCl gradient. The fractions