Secretory Production of Human Growth Hormone in *Escherichia coli* in presence of lactose as inducer using pelB signal sequence

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Abstract

A gene encoding the mature form of the human growth hormone (hGH) was fused to the secretion signal coding sequence, pelB, this hybrid gene was expressed in *E. coli* under the transcriptional control of bacteriophage T7 lac promoter/operator system. Studying the periplasmic protein pattern of the recombinant bacteria, showed that the recombinant bacteria allowed a partially succeeded secretion of hGH into the periplasmic space. Remaining of unprocessed pelB::hGH fusion-protein was also observed in the *E. coli* cytoplasmic space. Comparing to IPTG induction, we have also used lactose as natural inducer for over-expression of rhGH in the present system. Our results demonstrated that lactose is as effective as IPTG for inducing expression of recombinant hGH.

Introduction

Variation of parameters involved in expression of recombinant protein is a phenomenon commonly observed in designing strategies for genetically engineering proteins. Different permutation and combinations of vectors, promoters, signal sequences and regulatory signals have been tried for improving the quality and quantity of recombinant proteins.

It has been well documented that recombinant proteins expressed in *E. coli* can remain soluble in the cytoplasm, become sequestered in to inclusion bodies or be exerted across the cytoplasmic membrane to accumulate in the periplasmic space (Becker, 1996).

A variety of signal peptides from *E. coli* have been tried to achieve export of soluble hGH to the periplasmic space, for example ompA, heat labile enterotoxin chain B (LTB), Bacillus amiloliquefaciens natural protease signal sequence (npr) (Chang, 1987; Ghorpade, 1993; Goldstein 1990) and a lot of promoters also were used for this purpose such as T5, npr, ptrc and trc (Gray, 1989; Ghorpade, 1993).

But the T7 system comparing T7 promoter and T7 RNA polymerase is one of the most commonly used system for recombinant protein production in *Escherichia coli* (Tabor and Richardson, 1985; Studier and Moffit, 1986).

The need for a costly inducer like IPTG in the T7 system where the T7 RNA polymerase gene is under control of lac promoter (e.g. *E. coli* BL21 (λDE3)) increase the fermentation cost and is thus not preferred in the scale-up. In addition, for recombinant proteins of therapeutic pharmaceutical importance the presence of IPTG further complicates downstream processing. Considering this, the use of the lactose as natural analog of IPTG to induce T7 RNA polymerase synthesis in the T7 system is more preferable (Donovan, 1996).

Materials and methods

Strains and plasmids and expression vectors

The T7 based expression vector pET21 (Novagen) (ampR) was used for cloning the pelB-hGH. *E. coli* strains TG1 and BL21 (carrying the T7 RNA polymerase) were used as the host cell for cloning and expression respectively.
Growth conditions and induction of hGH expression

_E. coli_ cells harboring the recombinant plasmid were grown for 10-15 h in Luria Berteni medium (LB) with 100 g/ml of ampicillin freshly sub cultured in 50 ml shake flasks at 30°C with shaking at 200 rpm. When cultures reached OD₆₀₀ =1.8, cells were induced with 1 mM IPTG or lactose for 8 hour. In the case of sucrose when cultures reached OD₆₀₀ =1.8, bacteria were pelleted by centrifugation and resuspended in an equal volume of fresh LB medium containing 50 g/ml ampicillin. Lactose was added to the 0.001% final concentration and growth was continued at 30°C for 16 h. control cultures were induced with lactose without changing the medium.

Analysis of bacterial sample

Cell culture was centrifuged and the pellet was put on ice. The following samples were analyzed: (a) soluble periplasmic content: (b ) spheroplast pellet after isolating soluble periplasmic proteins. to isolate soluble periplasmic proteins, pellets were resuspended in 1% of initial volume of ice cold lysis buffer (Tris-HCl 0.2 M Ph 8.0, EDTA 0.5 Mm Ph 8.0, Sucrose 0.5 M), after 20 min on ice with shaking, 0.15% initial volume ice cold double distilled water was added and the incubation was continued for 30 min. the spheroplasts were centrifuged at 13000 rpm for 20 min and 4°C leaving the soluble periplasmic extract as the supernatant and spheroplast plus insoluble periplasmic material as the pellet.

SDS-PAGE and Western blotting

Sodium dodecyl sulfate-PAGE was performed according to Laemmli (Laemmli, 1970) using coomassie Blue G250 staining. Western blotting was performed using standard methods (Sambrook, 2001).

Results

Construction of pelB::hGH

Plasmid pZPH1 was constructed by fusion PCR (SOE-PCR) approach. Two PCR were performed by primer pET/pQE (CGGAATTCTCTAGAAATAATTTTGTT) as sense primer and pelB-hGH (CGAGATAGTGGTATAGTTGGGAAGGCCATCGCCGGCTGG) designed in reverse direction from the least 15 nucleotides of pelB signal sequence joined to the 20 nucleotides of mature hGH coding region, primer GH start II (TTCCCAACTTACCATACACT) and 190 that pET26 (Novagen) and A1 (pET21::hGH) as the template respectively. PCR product was joined to each other and ligated to pET21.

Periplasmic secretion of hGH

_E. coli_ cells harboring plasmid ZPH1 were induced for expression with IPTG fig (2). A band comparing with the standard hGH preparation was observed in the periplasmic fraction of the induced cells that could be immunoblotted with anti-hGH goat polyclonal sera (lane 4).The cytoplasmic cell extract of induced cells also showed a single bond corresponding to that of mature hGH and band of the unprocessed molecules was detected as the polyclonal antisera raised against hGH can detected the unprocessed molecules. hGH amount in periplasmic space was 6.9 μg/ml.

Effect of lactose as inducer for rhGH expression

Despite the fact that lactose is readily available in large quantities as a by product of the dairy industry, only a limited number of laboratory studies have examined conditions for using lactose to induce foreign protein expression from lac operator. This reflects the fact that optimizing conditions using lactose, as the inducer may be a more complex task than for...
IPTG due to the physiological response of the cell to the presence of the sugar and the fact that the cell metabolizes it. However, because of high cost of IPTG compared to lactose, the use of the latter may provide an inexpensive alternative means of induction foreign protein expression from the lac operator. IPTG is not only costly, but it may also toxic to humans so that its presence as a contaminant of the final purified protein product is problematic. Lactose is as effective as IPTG for inducing recombinant hGH using the T7 phage polymerase system controlled by lac operator. Expression of hGH using lactose as a inducer source yielded greater amounts of recombinant protein than was produced by 1 mM IPTG. While recombinant protein was produced during the log phase of batch growth with IPTG, high levels of product were produced with lactose during the late loge and stationary phase. We demonstrated that in our system lactose is better than IPTG. Our results are summarized in chart 1.

Fig. 1: (left) Design and molecular cloning of DNA coding the pelB signal sequence and hGH coding region.

Fig.2: (right) Analysis of hGH prepared from osmotic shock and spheroplast. Lane 1: BL21 spheroplast fraction, lane 2: pZPH1 spheroplast fraction, lane 4: pZPH1 periplasmic fraction, lane 3: BL21 periplasmic fraction and lane 5: standard hGH.
Chart 1: Effect of IPTG and lactose on production of hGH (g/ml). Lane 1: 0.1% lactose, lane 2: 0.01% lactose, lane 3: 0.001% lactose, lane 4: 0.0001% lactose and lane 5: 1mM IPTG

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