PUBLICATIONS
Extracellular expression and single step purification of recombinant *Escherichia coli* l-asparaginase II

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Received 19 April 2004, and in revised form 14 July 2004

Available online 28 August 2004

Abstract

l-Asparaginase (isoenzyme II) from *Escherichia coli* is an important therapeutic enzyme used in the treatment of leukemia. Extracellular expression of recombinant asparaginase was obtained by fusing the gene coding for asparaginase to an efficient pelB leader sequence and an N-terminal 6× histidine tag cloned under the T7lac promoter. Media composition and the induction strategy had a major influence on the specificity and efficiency of secretion of recombinant asparaginase. Induction of the cells with 0.1 mM IPTG at late log phase of growth in TB media resulted in fourfold higher extracellular activity in comparison to growing the cells in LB media followed by induction during the mid log phase. Using an optimized expression strategy a yield of 20,950 UI/L of recombinant asparaginase was obtained from the extracellular medium. The recombinant protein was purified from the culture supernatant in a single step using Ni-NTA affinity chromatography which gave an overall yield of 95 mg/L of purified protein, with a recovery of 86%. This is ~8-fold higher to the previously reported data in literature. The fluorescence spectra, analytical size exclusion chromatography, and the specific activity of the purified protein were observed to be similar to the native protein which demonstrated that the protein had folded properly and was present in its active tetramer form in the culture supernatant.

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Keywords: Recombinant asparaginase; Extracellular expression; *Escherichia coli*; Acute lymphoblastic leukemia

Secretory expression is an elegant solution to many common problems associated with recombinant protein production in *Escherichia coli*. Secretion results in few contaminating proteins as *E. coli* is not known to secrete many proteins into the culture medium [1,2]. There is no space limitation for the accumulation of the protein, moreover the cells need not be lysed or processed for extracting the desired protein all of which tremendously simplifies the downstream processing steps. The extracellular compartment is protease deficient which is important for proteins susceptible to proteolysis, the protein exported to the medium is mostly soluble, biologically active, and has an authentic N-terminus, it is relatively free from endotoxins and also results in minimization of the potentially harmful action of recombinant proteins against the host cells. Finally secretion facilitates proper folding of the proteins specially those requiring disulfide bridge formation, as it passes through a more favorable redox potential in the periplasmic space [1,3].

Several approaches have been adopted to promote the secretion of recombinant proteins by *E. coli* [2,3]. Utilizing the existing pathways for the truly secreted proteins in *E. coli* like hemolysin (Type I), pullanase/ *Klebsiella* system (Type II or the main terminal branch of the general secretory pathway), Yop production in *Yersenia* (Type III), pertusis toxin (Type IV), and Tat ABCE translocation pathway, have met with limited success. Though the export is protein specific the rates of export are low leading to low levels of extracellular expression [4,5]. Co-expression of proteins coding for
Optimization of extracellular production of recombinant asparaginase in Escherichia coli in shake-flask and bioreactor.

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Various host-vector combinations were tested to maximize the extracellular production of recombinant asparaginase in Escherichia coli. Expression of recombinant asparaginase fused to pelB leader sequence under the inducible T7lac promoter in BLR (DE3) host cells resulted in optimum extracellular production in shake-flasks. Fed-batch studies were carried out using this recombinant strain and an exponential feeding strategy was used to maintain a specific growth rate of 0.3 h(-1). To check the effect of the time of induction on expression, cultures were induced with 1 mM isopropyl-beta-D-thiogalactopyranoside at varying cell optical densities (OD(600): 33, 60, 90, 135). Although the specific product formation rates declined with increasing OD of induction, a maximum volumetric activity of 8.7x10(5) units 1(-1), corresponding to approximately 5.24 g 1(-1) of recombinant asparaginase, was obtained when induction was done at an OD(600) of 90. The recombinant protein was purified directly from the culture medium, using a rapid two-step purification strategy, which resulted in a recovery of approximately 70% and a specific activity of approximately 80% of that of the native enzyme.

PMID: 15660216 [PubMed - as supplied by publisher]