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Production of human calcitonin: multimeric fusion gene expression, cleavage, amidation and purification

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Background and Aims: The purpose of this study was to develop and optimize the production process for human calcitonin (hCT) in Escherichia coli. Calcitonin plays an important role in regulating calcium and phosphorus metabolism, decreasing blood calcium concentrations and inhibiting bone resorption. Hormone is widely used for the treatment of postmenopausal osteoporosis, Paget's disease of bone and bone pain.
Methods: Synthetic gene encoding hCalc3-Leu-Arg, a substrate for C-terminal amidation by carboxypeptidase Y (CPY) was cloned into NdeI/BglII sites of pET26b and transformed to BL21(DE3)(pDIA17). The recombinant protein was accumulated as inclusion bodies, after fermentation, the cells were harvested, disrupted by a homogenizer and suspended in 8M urea solution. The solubilized, citraconitated, S-sulfonated and polyhistidine-tagged protein was purified by immobilized metal affinity chromatography column and digested with trypsin to cleave into monomers of citraconilated and S-sulfonated (S-hCT-Leu-Arg). The subsequent decitraconilation was performed at low pH. S-hCT-Leu-Arg, isolated by HPLC and amidated by CPY.
Results: Constructed appropriate expression plasmid, which was stable during culture, expressed the polyhistidine tagged recombinant multimeric human calcitonin. The efficiency of the cleavage reaction is one of the most important factors in obtaining a desired peptide. Trypsin digestion occurs at the C-terminal side of Lys and Arg residues. To prevent cleavage at the Lys residues in the hCT sequence, citraconilation reaction was used on the c-amino groups of Lys residues and on the N-terminal a-amino group. Furthermore, introducing acidic groups into the fusion protein improved the solubility.
Conclusions: we attempted the pilot-plant production of hCT incorporating the above improvements. From 20 L fermentation, we obtained about 42 mg/L of recombinant hCT using a multimeric fusion protein expression system.
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Keywords: Human calcitonin; Recombinant multimeric protein; *E. coli*