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Expression and preparation of recombinant hepcidin in *Escherichia coli*

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Abstract

Hepcidin is a low-molecular-weight, highly disulfide bonded peptide relevant to small intestine iron absorption and body iron homeostasis. In this work, hepcidin was expressed in *Escherichia coli* as a 10.5 kDa fusion protein (His-hepcidin) with a N-terminal hexahistidine tag. The expressed His-hepcidin existed in the form of inclusion bodies and was purified by IMAC under denaturation condition. Since the fusion partner for hepcidin did not contain other cysteine residues, the formation of disulfide bonds was performed before the His-tag was removed. Then, the oxidized His-hepcidin monomer was separated from protein multimers through gel filtration. Following monomer refolding, hepcidin was cleaved from fusion protein by enterokinase and purified with reverse-phase chromatography. The recombinant hepcidin exhibited obvious antibacterial activity against *Bacillus subtilis*.

Keywords: Hepcidin; *Escherichia coli*; Inclusion body; His-tag

Hepcidin is a small cysteine-rich cationic peptide predominantly expressed in the liver and has been isolated from human blood ultrafiltrate and urine by two groups in search of novel antimicrobial peptides [1,2]. There are two predominant forms of hepcidin in urine which differed only by amino-terminal truncation and contained 20 and 25 amino acid residues, respectively. Hepcidin contains eight cysteine residues and four intramolecular disulfide bonds. Like other cysteine-rich antimicrobial peptides such as defensins [3], hepcidin exhibits obvious antibacterial and antifungal activity. In addition to its antimicrobial function, hepcidin plays an important role in the regulation of small intestine iron absorption and body iron homeostasis [4]. Hepcidin regulates cellular iron absorption through interacting with ferroportin [5]. Lack of hepcidin gene expression resulted in tissue iron overload, while transgenic mice expressing hepcidin had severe iron-deficiency anemia [6,7]. The mutant of hepcidin had relation with juvenile hemochromatosis [8]. Hepcidin mRNA levels were also associated with anemia, hypoxia, and inflammation [9]. Thus, this peptide may be medically applied in the treatment of various iron homeostasis disorders.

To elucidate biological function of hepcidin further and use it for other research, it is necessary to produce enough hepcidin through DNA recombinant technique. Although hepcidin can be separated from urine, its native concentration is very low [2]. And the chemical synthesis of hepcidin was not very easy[10]. To date, the expression and preparation of recombinant hepcidin has not been reported. Herein, we express 25 aa hepcidin as a fusion protein (His-hepcidin) having a 6× His-tag at its N-terminus in *Escherichia coli*. The fusion protein does not contain other cysteine residues except eight cysteine residues which are included in hepcidin. So, the formation of disulfide bonds was performed while hepcidin was still attached to its fusion partner. Then, hepcidin

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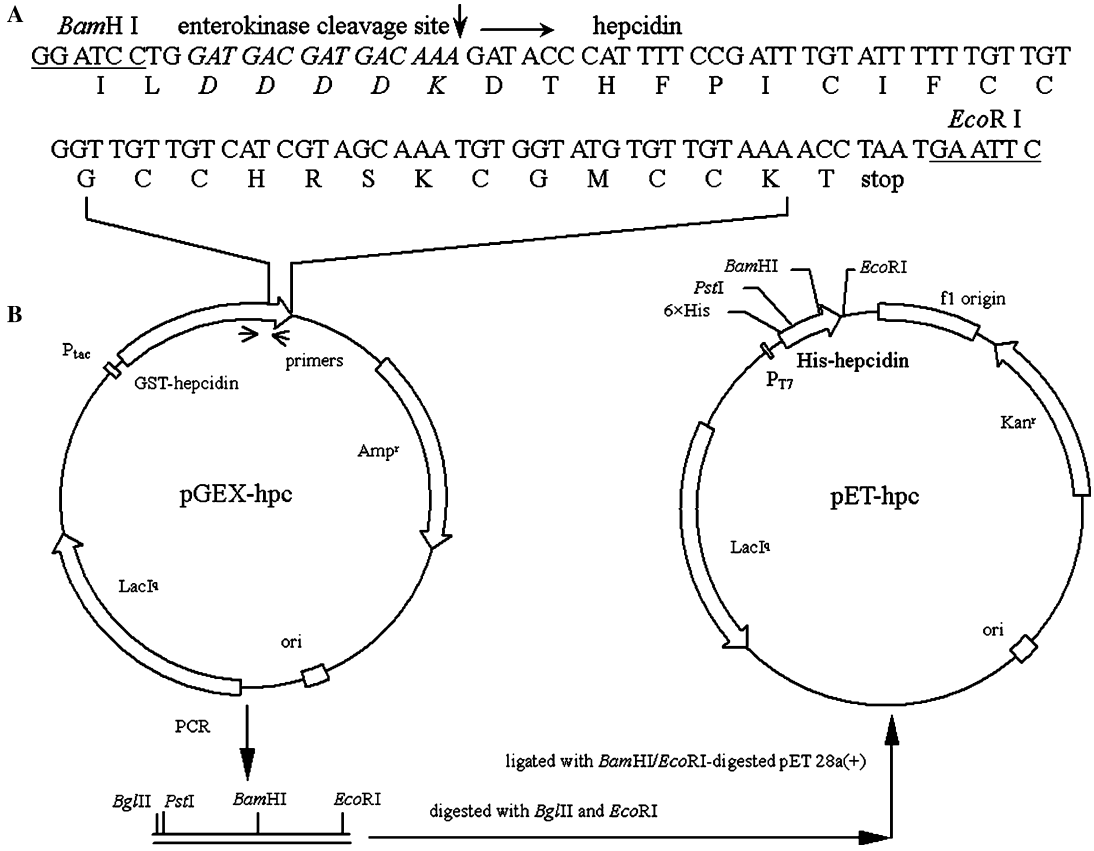

Fig. 1. (A) Chemically synthesized DNA fragment containing hepcidin sequence. (B) Construction of the expression vectors.

was cleaved from oxidized His-hepcidin monomer by enterokinase. This method also can be used for the preparation of other short peptides having one or more disulfide bonds such as cysteine-rich antimicrobial peptides.

Materials and methods

Strains

Cloning of plasmids was carried out in *E. coli* DH5a. *Escherichia coli* BL21(DE3) was used for protein expression.

Plasmid construction

According to codon preference in *E. coli*, a 108-bp fragment containing the coding sequence of enterokinase recognition site and hepcidin was synthesized, which is shown in Fig. 1. The fragment was inserted into pGEX-3X (Amersham Biosciences) through *Bam*HI and *Eco*RI site. The resulting pGEX-hpc was confirmed by PCR and DNA sequencing.

Plasmid pET-hpc was constructed on the basis of pGEX-hpc. A 217-nucleotide fragment was amplified from pGEX-hpc by PCR. The forward primer (5′-GAAGATCTGCAGTGTATATAGCATGGCCTTTG CAG-3′) introduced a *Bgl*II site (underlined) and a *Pst*I site (bold). The *Pst*I site which did not exist in pET 28a(+) was used to facilitate the identification of the recombinant plasmid. The designed reverse primer was 5′-GTCAGTCACGATGAATTCATTAGG-3′ in which the underlined site was *Eco*RI. The PCR product was digested with *Bgl*II and *Eco*RI, and was ligated into the *Bam*HI/*Eco*RI-digested pET 28a(+) (Novagen). Since *Bam*HI and *Eco*RI sites were adjacent in pET 28a(+), the *Bam*HI/*Eco*RI-digested vector fragment was dealt with CIAP before ligation. The fragment inserted into pET 28a(+) contained not only the chemically synthesized DNA sequence but also the coding sequence of some residues at the C-terminus of glutathione *S*-transferase (GST)[1]. The resulting pET-hpc was verified by restriction enzyme analysis and DNA sequencing.

Expression of GST-hepcidin and its absorption on glutathione–Sepharose 4B

A single colony of *E. coli* BL21(DE3) transformed with pGEX-hpc was inoculated into 2× YT medium containing 100 μg/ml ampicillin, and was cultured overnight at 37 °C. The overnight culture was inoculated

[1] *Abbreviations used:* GST, glutathione *S*-transferase; CV, column volume; CD, circular dichroism.