Expression of HspA-UreB Fusion Protein of *Helicobacter pylori* and Its Immunocompetence

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Abstract: Objective. To construct recombinant plasmid expressing HspA-UreB fusion protein of *H. pylori*, and to determine its immunocompetence. Methods. The *hspA* and *ureB* genes were amplified by PCR from *H. pylori* strain MEL-HP27 isolated in Zhengzhou and cloned directly into vector pET30a, and the recombinant plasmid was then transformed into *E. coli* BL21DE3. The recombinant plasmid was induced to express fusion protein HspA-UreB in *E. coli* by isopropylthio-β-D-galactoside (IPTG). The protein was analyzed by SDS-PAGE, purified by Ni²⁺ affinity chromatography, and immunized the mice. The immunoreactivity of the fusion protein were analyzed by Western blot. Results. In comparison with the reported corresponding sequence from Genbank, the nucleotide sequence homologies of the cloned *hspA* and *ureB* genes were 95.20 - 97.48% and 96.08 - 98.30%, and their putative amino acid sequence homologies were 95.76 - 97.46% and 98.77 - 99.82% for the two genes, respectively. The results of SDS-PAGE and optical density scanning indicated that the fusion protein was expressed by pET30a-hspA-ureB-BL21DE3 as a protein with Mr 82,100 of molecular weight and was 21% of the total bacterial proteins. The purity of fusion protein was 91%, and could be recognized by the serum from *H. pylori* infected patients and mice immunized with purified HspA-UreB fusion protein. Conclusion. A recombinant plasmid expressing fusion protein HspA-UreB of *H. pylori* was constructed and identified with good immunocompetence, and it suggested that HspA-UreB might be a potential vaccine antigen for controlling and treating *H. pylori* infection. [Life Science Journal. 2006; 3(2):21 - 26] (ISSN: 1097 - 8135).

Keywords: *Helicobacter pylori*; HspA-UreB; immunocompetence

Abbreviations: HapA: *Helicobacter pylori* adhesion A; HspA: heat shock protein subunit A; Lpp20: lipoprotein 20; NAP: neutrophil-activating protein; Omp: outer membrane protein; UreB: urease subunit B; VacA: vacuolating cytotoxin A

1 Introduction

*Helicobacter pylori* (*H. pylori*) infection is a major cause of chronic active gastritis and most peptic ulcer diseases¹-³, and also closely related to gastric cancers⁴,⁵. This microorganism has been categorized as class I carcinogen by the World Health Organization⁶. For this reason, successful eradication of *H. pylori* may be an important goal for this study. Currently, the treatment for *H. pylori* infection involves antibiotic therapy, but this has some disadvantages such as increasing the expense and strains resistance⁷,⁸. An alternative approach is to develop a vaccine, which could eradicate *H. pylori* infection⁹,¹⁰. Selection of antigenic epitope is critical in developing *H. pylori* vaccine. The majority of studies attempting to produce a vaccine have focused on urease enzyme¹¹,¹², heat shock protein¹³,¹⁴ and vacuolating cytotoxin¹⁵. The protection afforded by a single antigen is not enough¹⁶, but the two kinds antigen HspA and UreB combined could provide 100% protection for mice from being infected with *H. pylori*.

In this study, the recombinant expression system for HspA-UreB fusion protein of *H. pylori* was constructed. Immunoreactivity and immunogenicity of HspA-UreB fusion protein were further examined. The results of this study may contribute to the development of *H. pylori* vaccines.

2 Materials and Methods

2.1 Materials

A clinical strain of *H. pylori*, MEL-HP27 was isolated from a patient with chronic gastritis. Bacterial strain BL21 (DE3) and plasmid pET-30a were purchased from Novagen (Madison WI, USA). Primers for PCR amplification and restriction endonucleases Sal I, Xho I, EcoR I and T4
DNA ligase were purchased from Sangon (Shanghai, China). The Pyrobos™ high fidelity DNA polymerase and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Takara Company (Dalian, Jilin, China). Goat anti-mouse and goat anti-human IgG-HRP were purchased from Bangding Bioengineering company (Beijing, China). The nucleotide sequences were compared with the published sequences.

2.2 Construction of expression system pET30a-hspA-ureB-BL21 (DE3)

Genomic DNA of MEL-HP27 was extracted by conventional phenol-chloroform method. Oligonucleotide primers were designed based on the corresponding genomic sequence of international standard strain NCTC11637. The sequence of hspA sense primer with an endonuclease site of EcoRI was 5'-CCC GAA TTC ATG AAG TTT CAA CCA TTA-3'. The sequence of hspA antisense primer with an endonuclease site of SalI was 5'-CGC GTC GAC GTG TTT GTT ATG AC-3'. The sequence of ureB sense primer with an endonuclease site of SalI was 5'-CCG GTC GAC AAA AAG ATT AGC AGA AAA G-3'. The sequence of ureB antisense primer with an endonuclease site of XhoI was 5'-CGC CTC GAG CTA GAA AAT GCT AAA GAG-3'. PCR was performed with the hot start method.

The DNA ligase were purchased from Sangon (Shanghai, China). The Pyrobos™ high fidelity DNA polymerase and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Takara Company (Dalian, Jilin, China). Goat anti-mouse and goat anti-human IgG-HRP were purchased from Bangding Bioengineering company (Beijing, China). The mice were provided by the Center of Experimental Animal of Henan (Zhengzhou, Henan, China).

2.3 Expression, purification and identification of fusion proteins

The recombinant strains were incubated overnight at 37°C while shaking in 5 ml LB medium with 100 μg/mL kanamycin, and the cell grew until the optical density at 600 nm reached 0.4—0.6. IPTG was added to a final concentration of 0.3 mmol/L. The cells growing for 4 h after induction were harvested by centrifugation at 4,000 g for 20 min. The molecular weight and output of HspA-UreB fusion protein were examined by SDS-PAGE. The serum of patient infected with H pylori and commercial goat-human HRP-IgG were used as the first and second antibodies to identify the immunoactivity of HspA-UreB. The recombinant E. coli cells growing in 50 ml LB medium with kanamycine for 3 h induced by IPTG harvested by centrifugation at 4,000 g for 20 min. The bacterial pellets were washed and centrifuged at 12,000 g for 15 min. The recombinant HspA-UreB fusion protein was collected by Ni-NTA affinity chromatography and analyzed by electrophoresis in a 10% polyacrylamide gel.

3 Results

3.1 PCR amplification of H pylori hspA and ureB genes

Target fragments of hspA and ureB genes with expected sizes amplified from DNA template of H pylori MEL-HP27 were shown in Figure 1.

3.2 Identification of recombinant plasmid

After extracting plasmids DNA from recombinant E. coli strains, the recombinant plasmids were digested by EcoRI or XhoI, and by EcoRI and XhoI simultaneously, then the digestion products were visualized on 10 g/L agarose gel electrophoresis (Figure 2). It demonstrated that recombinant plasmid contained the objective fusion gene.

3.3 Nucleotide sequence analysis

The nucleotide sequences of hspA and ureB gene in pET30a-hspA-ureB were listed in Figure 3 and Figure 4. The homologies of nucleotide and putative amino acid sequences of the cloned hspA gene compared with the published hspA sequences were
from 95.20% to 97.48% and from 95.20% to 97.46%, respectively (Table 1). The homologies of nucleotide and putative amino acid sequences of the cloned ureB gene were from 96.08% to 98.30% and from 98.77% to 99.65% (Table 2).

3.4 Expression, purification and identification of HspA-UreB fusion protein

IPTG at concentration of 0.3 mmol/L efficiently induced the expression of HspA-UreB in the pET30a-hspa-ureB-Bl21DE3 system. SDS-PAGE analysis showed that the clearly identifiable band with Mr 82,100 highly expressed fusion proteins, which was similar to that predicted.

The output of HspA-UreB fusion protein was approximate 21% of the total bacterial proteins (Figure 5). Among them, soluble substance accounted for 30% of supernatant. HspA-UreB fusion protein was further purified with Ni-NTA column, its final purity was 91%. Western blot also showed an identifiable blot band with Mr 82,100.

3.5 Antigenicity of recombinant HspA-UreB fusion protein

An immunized reacting band with the weight of Mr 82,100 appeared in the Western blot (Figure 6) in which the purified HspA-UreB fusion protein reacted against the serum of mouse immunized by HspA-UreB.

Table 1. Homology comparison of H pylori hspA gene sequences

<table>
<thead>
<tr>
<th>H pylori strains compared with MEL-HP27</th>
<th>Different base pair</th>
<th>Homology of nucleotide</th>
<th>Different base pair</th>
<th>Homology of amino acid</th>
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<tbody>
<tr>
<td>NCTC11637</td>
<td>9</td>
<td>97.48%</td>
<td>3</td>
<td>97.46%</td>
</tr>
<tr>
<td>26695</td>
<td>11</td>
<td>96.89%</td>
<td>3</td>
<td>97.46%</td>
</tr>
<tr>
<td>CH-CTX1</td>
<td>17</td>
<td>95.20%</td>
<td>5</td>
<td>95.76%</td>
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<tr>
<td>J99</td>
<td>10</td>
<td>97.18%</td>
<td>4</td>
<td>96.61%</td>
</tr>
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</table>

Table 2. Homology comparison of H pylori ureB gene sequences

<table>
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<tr>
<th>H pylori strains compared with MEL-HP27</th>
<th>Different base pair</th>
<th>Homology of nucleotide</th>
<th>Different base pair</th>
<th>Homology of amino acid</th>
</tr>
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<tbody>
<tr>
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<td>97.67%</td>
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<td>HPK5</td>
<td>29</td>
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<td>1</td>
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<tr>
<td>26695</td>
<td>46</td>
<td>97.31%</td>
<td>6</td>
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<tr>
<td>HP031</td>
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<td>97.13%</td>
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</tr>
<tr>
<td>J99</td>
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<td>96.08%</td>
<td>2</td>
<td>99.65%</td>
</tr>
<tr>
<td>CH-CTX1</td>
<td>51</td>
<td>97.01%</td>
<td>7</td>
<td>98.77%</td>
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</tbody>
</table>
Expression of HspA-UreB Fusion Protein

Figure 3. hspA nucleotide sequence of H pylori strain MEL-HP27

Figure 4. ureB nucleotide sequence of H pylori strain MEL-HP27

Discussion

Immune protection function of several H pylori antigens has been studied and investigated, such as UreB[17,18], HspA[19], VacA[15], Catalase[20,21]. Several studies demonstrated that three of H pylori antigens, such as Lpp20[22,23], NAP[24,25], HpaA[26], Omp[27] are also excellent and ideal antigens that can be potentially used for the development of H pylori vaccine. However, some researches indicated that immune protection function of combined antigens was better than single antigen. Recently, some researchers study polyanitgen genetic engineering vaccine of H pylori in China[27,28]. This research selected two kinds of antigens with effective immunization: HspA and UreB, to construct the expression system for hspA-ureB fusion gene, and to determine the immunogenicity and immunoreactivity of HspA-UreB fusion protein. UreB, used as a candidate antigen for H pylori genetic engineering vaccine, has advantages of high sequence conservation, high frequency of distribution, large expression in different isolates, strong antigenicity due to its big molecular mass, and granular structure and exposure on the
surface of bacteria\cite{29,30}. HspA is another candidate antigen for \textit{H pylori} vaccine, which is termed as "molecular chaperone" because they assist in post-translational assembly, secretion, and stability of oligomeric protein structures\cite{31}.

Figure 5. SDS-PAGE analysis of HspA-UreB induced by IPTG at different time.
Lane 1: molecular weight marker; Lanes 2-5: BL21 (DE3) with \textit{pET30a-hspA-ureB} induced for 4, 3, 2 and 1 h; Lane 6: BL21 (DE3) with \textit{pET-hspA-ureB} uninduced for 4 h; Lane 7: BL21 (DE3) with \textit{pET30a} induced for 4 h.

Figure 6. Western blot result of mouse serum against recombinant HspA-UreB.
Lane A1: BL21 (DE3) with \textit{pET30a-hspA-ureB} uninduced for 4 h; Lane A2: BL21 (DE3) with \textit{pET-hspA-ureB} induced for 4 h; Lane A3: BL21 (DE3) with \textit{pET30a} induced for 4 h; Lane B1: unpurified HspA-UreB; Lane B2: purified HspA-UreB.

In this study, the recombinant plasmids expressing HspA-UreB fusion gene of \textit{H pylori} were constructed. The \textit{hspA} gene cloned from \textit{H pylori} strain MEL-HP27 showed high homologies of the nucleotide and putative amino acid sequences compared with five published corresponding sequences (Table 2). Similarly, the homologies of nucleotide and putative amino acid sequences of the clone \textit{ureB} gene from \textit{H pylori} strain MEL-HP27 were quite high when compared with the published corresponding sequences (Table 2).

The results of SDS-PAGE demonstrated that the constructed expression system \textit{pET30a-hspA-ureB-BL21 (DE3)} efficiently produced the target fusion protein. The most output of fusion protein HspA-UreB within whole cell protein was about 21% of the total bacterial proteins, which is beneficial to industrial production.

Western blot assay was performed in this study to confirm that the purified fusion protein HspA-UreB could be recognized by the serum from patient infected with \textit{H pylori} and also be recognized by the sera from mice immunized with purified fusion protein. HspA-UreB exhibited favorable immunogenicity and immunoreactivity.

In conclusion, HspA-UreB is excellent and ideal candidate antigen that can be potentially used for the development of \textit{H pylori} vaccine.

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Received March 10, 2006