Available online at www.sciencedirect.com
SCIENCE DIRECT®
Peptides 26 (2005) 1213–1218
PEPTIDES
www.elsevier.com/locate/peptides

High-level expression of human TFF3 in *Escherichia coli*

Haibo Wang, Yuanpeng Tong, Ming Fang, Binggen Ru *

Department of Biochemistry and Molecular Biology, National Laboratory and Protein Engineering, College of Life Science, Peking University, Beijing 100871, PR China

Available online 17 May 2005

Abstract

A strategy for expression and purification of recombinant N-terminal human trefoil factor family-domain peptide 3 (hTFF3) in *Escherichia coli* was established. The gene of hTFF3 was synthesized to substitute the low-usage condons with corresponding high-usage synonymous condons. At the same time, the signal peptide of DsbC was added to the N-terminus of the hTFF3 gene. The mature recombinant hTFF3 was located in the periplasm of *E. coli*, which can be released by sonication. The protein was further purified by a two-step cation exchange chromatography mentod. The yield is about 14–15 mg/l of culture. The biological activity of purified hTFF3 was analyzed by cell-based apoptosis assay, which shows that the recombinant hTFF3 is biologically active.

Keywords: Intestinal trefoil factor (ITF); Trefoil factor family-domain peptide 3 (TFF3); Osmotic shock; Two-step ion exchange chromatography

1. Introduction

The mammalian trefoil factor family-domain peptides (TFFs) include three members: TFF1/pS2, TFF2/SP and TFF3/ITF. All three genes are clustered on the chromosome 21q22.3 [7,16]. They contain one or two copies of conservative trefoil motif, $CX_9CX_9CX_4CCX_9WCF$, in the configuration: [20,22], The motif forms a secondary structure of a three-loop domain with three cysteine-cysteine bonds in the 1–5, 2–4, and 3–6 configuration [2,6,19]. TFF1 and TFF3 also have a seventh cysteine residue, which permits homodimer formation. The TFFs are mainly secreted by mucin secreting epithelial cells lining the gastrointestinal tract and have a close association with mucins. They are mainly secreted into the intestinal tract, and help to protect and repair the mucosal surface of the gastrointestinal tract [13]. TFFs have also been shown to inhibit the apoptosis [18] of epithelial cells and promote the migration of epithelial cells around mucasol wound to cove the breaches [3–5,9,15]. Therefore, TFF3 plays an important role in protection and reconstitution of colonic mucosa.

The hTFF3 has been expressed by our research group with the GST-fusion system in *Escherichia coli* and in secreted form in *Pichia pastoris* [10,24]. The expression yields of are 3 and 20 mg/l, respectiely. But the N -terminus contains unnatural sequences. Here we describe a method to express recombinant TFF3 with natural N-terminus in the *E. coli* system.

2. Materials and method

2.1. Chemicals, media, E. coli, tools, and cell

Peptone, and Yeast Extract were obtained from Gibco BRL. Chemicals were purchased from Amresco. *E. coli* strain DH5 α and BL21 were kept in our group. The expression plasmid pQE30 was obtained from QIAGEN. HCT116 cell was obtained from ATCC. Chromatography system was purchased from Amersham Bioscinces.

2.2. Synthesize ofcDNA sTFF3

In cDNA of hTFF3 there are some low-usage condons for *E. coli*, such as ^{52}AGG, ^{70}CCC, ^{82}CCC, ^{100}CGG, ^{118}TCC, ^{121}AGG, ^{127}CCT, ^{130}GGA, ^{136}CCT, ^{151}CCC. They reduced

* Corresponding author. Tel.: +86 10 62751842; fax: +86 10 62751842.
E-mail address: rulab@pku.edu.cn (B. Ru).

0196-9781/$ – see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.peptides.2005.04.012

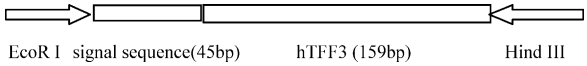

Fig. 1. Structure of cDNA sTFF3.

the expression of hTFF3 in *E. coli* largely. Since the cDNA of hTFF3 is not long (only 159 bp), so it is viable synthesize the complete cDNA of hTFF3 to substitute the low-usage condons with corresponding high-usage synonymous condons. At the same time the cDNA of signal peptide of DsbC was synthesized at the N-terminus of the modified cDNA of hTFF3. Two restrictive sites, BamH I and Hind III, were added to the ends of the synthesized cDNA. The sequence of sTFF3 is:

5′-cgcggatccatgaagaaaggttttatgctgttcactctgctggctgcgttttctg-
gcttcgcacaggctgaagaatacgttggtctgtctgcta
accagtgcgctgttccggcgaaagaccgtgtagactgcggctacccgcacgtta-
ccccgaaagaatgtaacaaccgcggttgctgtttcgatt
cccgtatcccgggcgtaccgtggtgcttcaaaccgctgcaggaggcagaatgc-
accttcuaauagaagcttggg-3′.

The structure of the cDNA of sTFF3 is shown in Fig. 1.

2.3. Tranformation of E. coli DH5α and BL21

The artificially synthesized cDNA was digested with BamH I and Hind III, and ligated into the vector pQE30. The recombinant plasmid was named pQEsTFF3, which was transformed into competent *E. coli* DH5α.The transformants were selected on LB agar plates in the presence of 100 μg/ml of ampicillin. The recombinant plasmid pQEsTFF3 was sequenced to ensure its 100% identity with the expected nucleotide sequence. The correct pQEsTFF3 was prepared from DH5α and transformed into the competent *E. coli* strain BL21.

2.4. Optimizing expression conditions of hTFF3

The hTFF3 transformant was grown in LB at 37 °C for 14 h and amplified by 1:50–2 × YT broth for 2.5 h with shaking. Then the cells were induced by different concentrations of isopropyl-β-D-thiogalactoside (IPTG) (0.01, 0.05, 0.1, 0.2, 0.4 mmol/l) for 8 h, or induced by 0.1 mmol/l IPTG for different time (2, 4, 6, 8 and 10 h). The expression of hTFF3 was analyzed by SDS gel electrophoresis.

2.5. Expression of hTFF3

The hTFF3 transformant was grown in 20 ml LB at 37 °C for 14 h, then was added into 1 l 2 × YT for 2.5 h until its OD_{600} reaches 0.5 ∼ 1. IPTG was added into culture to final concentration of 0.2 mmol/l. After induction for 6 h, the cells were pelleted by centrifugation at 6000 × *g* for 10 min.

2.6. Purification of recombinant hTFF3

2.6.1. Release of content in the periplasm by osmotic shock

Cell pellet was resuspended in 400 ml buffer (20%sucrose, 30 mmol/l Tris-HCl and 2 mmol/l Na_2EDTA, pH 7.5). After a static incubation at room temperature for 15 min, the osmotically fragile cells were harvested by centrifugation at 13,000 × *g* for 15 min. The supernatant was removed and the cell pellet was resuspended in 400 ml distilled H_2O (4 °C) and incubated with gentle agitation at 4 °C for 15 min. Following centrifugation at 13,000 × *g* for 15 min at 4 °C, the supernatant, as the periplasm fraction, was recovered. The supernatant and the precipitate were analyzed by SDS gel electrophoresis.

2.6.2. Release of content in the periplasm sonication

Cell pellet was resuspended in 80 ml phosphate-buffered saline (PBS, 140 mmol/l NaCl, 2.7 mmol/l KCl, 10 mmol/l Na_2HPO_4, 1.8 mmol/l KH_2PO_4, pH 7.3). The re-suspended cells were disrupted by sonication at 50 W for 20–15 s cycles with a 1/8 in. diameter microtip (Xinzhi Ultrasonics Corporation, Zhejiang, China). Following adding 0.8 ml Triton-X 100, the broth was softly churned for 30 min at 4 °C. The suspension was centrifuged at 16,000 × *g* for 30 min at 4 °C. The supernatant containing hTFF3 was collected to store at −20 °C for usage.

2.6.3. Two-step cation exchange chromatography for the purification of hTFF3

The supernatant was adjusted to pH 5.2, a little higher than the p*I* 4.98 of hTFF3, with 1 mol/l formic acid and some water added to reduce the conductivity of the solution (less than 6 mS). The resulting solution was centrifuged at 16,000 × *g* for 30 min at 4 °C, and the supernatant was loaded onto SP-Sepharose Fast Flow Column (Amersham), (2.0 cm × 10 cm). Prior to application, the column was equilibrated with buffer A: 20 mmol/l formic acid, pH 5.2. The solution penetrating the column was collected and adjusted to pH 3.5, lower than the p*I* of hTFF3, with 1 mol/l formic acid. The resulting solution was centrifuged at 16,000 × *g* for 30 min at 4 °C, and the supernatant was loaded onto the same SP column. Prior to application, the column was washed by 0.2 mM NaOH 50 ml and equilibrated with buffer B: 20 mmol/l formic acid, pH 3.4. After application the column was washed with buffer B 100 ml. hTFF3 was eluted from the column by a linear gradient between 75 ml buffer B and 75 ml buffer B containing 1 mol/l NaCl. The flow rate of equilibration, wash and elution was 2 ml/min. It was demonstrated by SDS gel electrophoresis that hTFF3 was in the solution corresponding the second peak, and the elute was collected and frozen-dry. Thereafter the frozen-dry sample was solved by 2 ml buffer C: NH_4HCO_3 10 mmol/l and passed Sephacryl S-100 column (Amersham) (2 cm × 60 cm). hTFF3 was eluted from the column by buffer C. The flow rate of elution was 0.5 ml/min. The second elution peak containing hTFF3,