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# Soluble expression and one-step purification of a neurotoxin Huwentoxin-I in *Escherichia coli*

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ABSTRACT

Huwentoxin-I (HWTX-I) is a small 33-amino acid neurotoxin from the venom of the Chinese bird spider *Ornithoctonus huwena*. HWTX-I selectively blocks N-type voltage-sensitive calcium channels (N-VSCCs) and has great potential for clinical application as a novel analgesic without inducing drug tolerance. However, there are still many unsolved issues for this peptide, such as its clinical efficacy in analgesia, anesthesia, and even its potential role in drug rehabilitation. Therefore, large amounts of active recombinant HWTX-I are urgently needed. In this report, we describe a novel and efficient way to produce large amounts of the valuable form in *Escherichia coli*. HWTX-I was expressed in soluble form as an N-terminal intein fusion product. After affinity purification, a pH shift-induced self-cleavage of the intein released HWTX-I, resulting in a single-column purification of the target protein. The whole-cell patch clamp assay showed that purified HWTX-I has activity similar to another commercialized N-VSCC blocker ω-conotoxin MVIIA. Production of HWTX-I by this method has the major advantages of high efficiency and low cost.


## Introduction

Huwentoxin-I (HWTX-I),[1] the most abundant toxic component in the venom of the Chinese bird spider *Ornithoctonus huwena*, consists of 33-amino acid residues (ACKGVFDACTPGKNECCPNRV CSDKHKWCKWKL). HWTX-I reversibly obstructs neuromuscular transmission, blocks N-type voltage-sensitive calcium channels (N-VSCCs), and alleviates neuropathic pain after epidural administration in an animal model [1–4]. Distinct from conventional analgesics, drug tolerance to HWTX-I does not develop [5]. These properties suggest that HWTX-I may be developed as a new and effective pain-killer for clinical application. ω-Conotoxin MVIIA, similar to HWTX-I, is a small 25-amino acid peptide from the venom of the marine cone snail *Conus magus* and also blocks N-VSCCs [6]. Comparison of their three-dimensional structures shows that these neurotoxic peptides share the structural motif of anti-parallel β-sheets stabilized by three disulfide bridges [7,8]. Intrathecal administration of HWTX-I is as effective as ω-conotoxin MVIIA in a rat model using the formalin test [5]. ω-Conotoxin MVIIA, known as ziconotide, has been developed into a commercial drug by Elan Corporation [9]. It was approved for sale by the U.S. Food and Drug Administration in December, 2004, and by the European Commission in February, 2005. In this regard, HWTX-I may also have great potential for development as a novel analgesic in clinical settings.

Recently most of the HWTX-I used in studies has been in the natural form purified from spider venom, but the amounts are quite limited. Although several protein expression systems have been tried, currently no efficient recombinant techniques are available to produce enough of the neurotoxin. Recombinant HWTX-I expressed in insect cells has biological activity similar to natural HWTX-I, but the expression level is low, and it is associated with high cost issues [10]. Production of HWTX-I in *Escherichia coli* using GST tag also results in very low yields. Proteolytic cleavage and the renaturation process require two additional purification steps, which cause more than 70% loss of expressed HWTX-I [11].

In this study, we established a new expression and purification method for producing large amounts of active HWTX-I in *E. coli*.

## Materials and methods

### *Recombinant plasmid construction*

The *hwtx-I* gene sequence used for protein expression was designed based on the amino acid sequence obtained from GenBank (Accession No. AAB28456) and adapted to the *E. coli* codon usage preference. 5′ and 3′ nucleotides corresponding to the *hwtx-I* sequence were synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China) as follows (Sap I and BamH I sites underlined, stop codon in bold). 5′ strand: 5′-AACGCGTGCAAAGGCGTGTTTGATGCGTGCACCCCGGGCAAAAAC

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[1] *Abbreviations used:* HWTX-I, Huwentoxin-I; N-VSCCs, N-type voltage-sensitive calcium channels; LB, Luria–Bertani; IPTG, isopropyl-beta-D-thiogalactopyranoside; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride.

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GAATGCTGCCCGAACCGTGTGTGCAGCGATAAACATAAATGGTGCAA ATGGAAACTG**TAA**G-3′; 3′ strand: 5′-GATCC**TTA**CAGTTTCCATTTG CACCATTTATGTTTATCGCTGCACACACGGTTCGGGCAGCATTCGTTTT TGCCCGGGGTGCACGCATCAAACACGCCTTTGCACGC-3′.

The 5′ and 3′ strands coding for HWTX-I were dissolved in water and annealed as follows: 95 °C 3 min, ramp 0.2 °C/sec to 50 °C, 50 °C 2 min. The *pTWIN1* plasmid (NEB, Ipswich, MA,USA) was cut with Sap I and BamH I restriction enzymes (NEB) and then the annealed double strands were inserted between the restriction sites to construct the *HWTX-I:pTWIN1* expression vector (Fig. 1). Verification of the *hwtx-I* sequence insertion was confirmed by DNA sequencing (Bioasia, Beijing, China).

*Expression and purification of HWTX-I*

The expression vector *HWTX-I:pTWIN1* was transformed into *E. coli* strain BL21 and the transformants were grown at 37 °C in Luria–Bertani (LB) medium containing 50 μg/ml ampicillin with shaking until the $OD_{600}$ reached 0.5. Protein expression was induced by adding isopropyl-beta-D-thiogalactopyranoside (IPTG) to a final concentration of 0.4 mM for 3 h at 37 °C with shaking. The cell pellet from a 1 L culture was resuspended in 100 mL ice-cold column buffer (20 mM Tris–HCl pH 8.5, 0.5 M NaCl, 1 mM EDTA) and ultrasonicated on ice. The cell lysate was then centrifuged and the supernatant loaded onto the chitin column (NEB). The column was then washed with 30 bed volumes of column buffer to wash away nonspecific protein binding. Activation of the cleavage step was conducted by quickly flushing the column with 3 bed volumes of the cleavage buffer (20 mM Tris–HCl, pH 7.0, 0.5 M NaCl, 1 mM EDTA) and storing the flow through at 4 °C for 48 h. Recombinant HWTX-I was eluted by adding 3 bed volumes of column buffer and dialyzing into distilled water at 4 °C for 24 h, changing to new distilled water every 8 h, prior to lyophilization.

The expression level and purification efficiency of HWTX-I were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (15% gel). The presence and purity of HWTX-I was further confirmed by Western blot analysis. The proteins separated on the gel were transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) that was blocked with TBS (20 mM Tris–HCl pH 7.5, 0.15 M NaCl) containing 5% dried milk powder, and then probed with 1:3000 diluted poly-

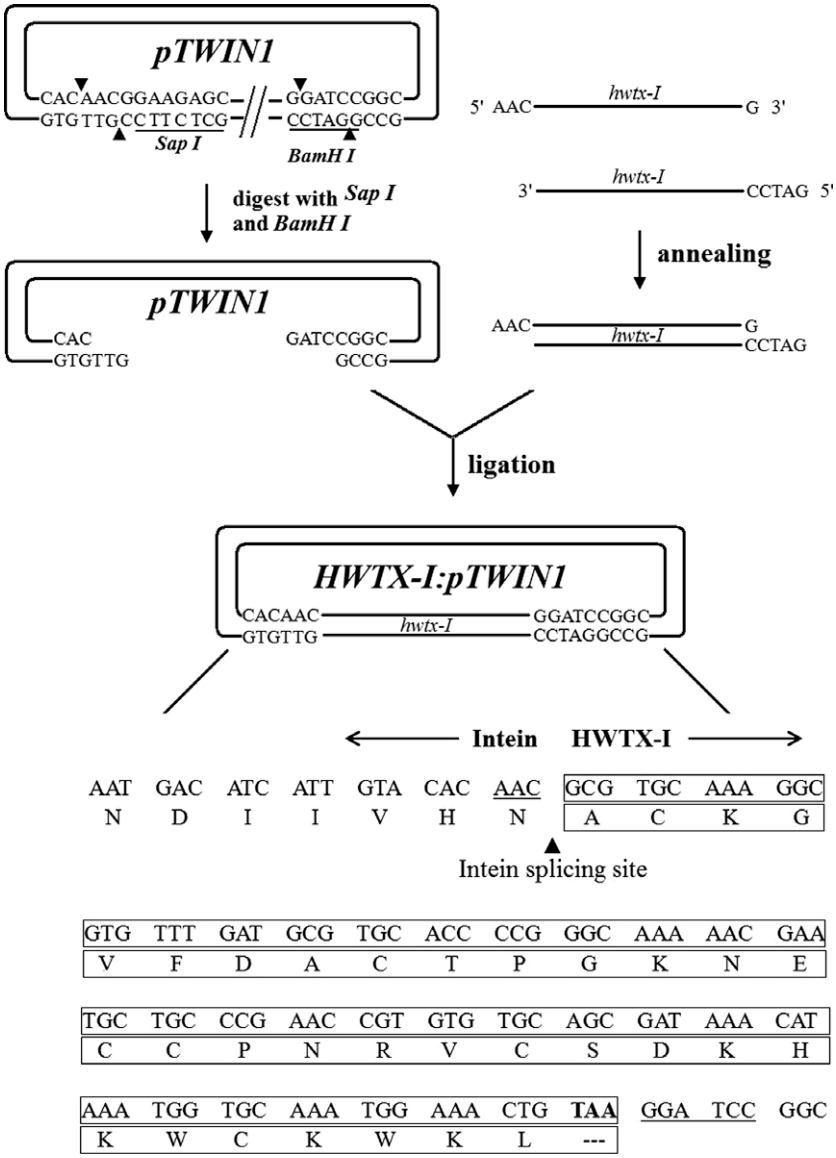


**Fig. 1.** Construction of *HWTX-I:pTWIN1* expression vector.