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Short sequence report

cDNA sequence encoding an antimicrobial peptide of chelonianin from the tiger shrimp *Penaeus monodon*

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Chelonianin is a member of the prophenoloxidase system family which includes prophenoloxidase, coagulation factor G-β chain precursor, factor D, Masquarade-like protease, transglutaminase (TGase), clottable protein, and eight types of protease inhibitors. Human mucous fluids, such as seminal plasma, cervical mucous, bronchial and nasal secretions, and tears, contain acid-stable proteinase inhibitors to inhibit pathogens [1]. The chelonianin group contains only one member, secretory leucocyte protease inhibitor (SLPI) [2]. SLPI is a 107-amino acid residue peptide that has the physiological function of protecting the mucosal epidermis against degradation by liberated proteolytic enzymes. Its function is the same as that of elafin [3,4]. SLPI and elafin share 40% homology and eight key cysteine residues [5]. SLPI and elafin are both expressed in the cervix and endometrium of the genital tract which must be protected from infection; these antimicrobial and anti-inflammatory molecules comprise part of the innate immune defence in mammals [6–8]. The main function of chelonianin in crustaceans is unknown, but it contains a motif with known proteinase inhibitors of the mammalian chelonianin family. The present paper shows the sequence of the chelonianin gene from *Penaeus monodon*.

Shrimp (*P. monodon*), purchased from a shrimp farm, weighed 5–10 g and were maintained in an aquarium with aeration for 14 days. Then, whole shrimp were immediately frozen in liquid nitrogen and homogenised in extraction buffer. Total RNA was isolated from the all shrimp bodies following the manufacturer's protocols (Ultraspectm-II RNA isolation system; Biotecx Laboratories, USA). Primers corresponding to sequences in comparison to chelonianin of scorpion (*Mesobuthus gibbosus*), kuruma shrimp (*Marsupenaeus japonicus*), and Pacific white shrimp (*Litopenaeus vannamei*) (accession nos. CB334114, AU176278, and BE188608, respectively) were then designed as a pair of primers. These PCR

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primers (p1 and p2) were used in the PCR to amplify the partial fragment of cDNA encoding the chelonianin gene of *P. monodon*. First-strand cDNA was synthesised for 60 min. These PCR primers (p1 and p2) were also used in the PCR to amplify the partial fragment of cDNA encoding the crustin gene of *P. monodon*. First-strand cDNA was synthesised for 60 min at 55 °C and then 15 min at 65 °C in a 20-μl reaction mixture containing 5 μg total RNA and 1 μg reverse primer (p2). Then 35 cycles of PCR amplifications were performed in 50 μl of reaction buffer, containing 5 μl of the first-strand cDNA reaction solution and 1 μg each of the forward (p1) and reverse (p2) primers (94 °C for 60 s, 55 °C for 120 s, and 72 °C for 120 s for 35 cycles). After the PCR reaction, the RT-PCR products were ligated with a topo ligation kit and sequenced to check whether the insertion was correct or not.

Primer oligonucleotides were prepared by a DNA synthesiser from Mission Biotechnology Company (Taipei, Taiwan). The sequences were as follows: p2, 5′-gctcagcaaagcacaggatctgattccc; and p1, 5′-ttccgtaaggaggaaccacagccaggc. The RT-PCR clones were randomly selected for sequencing on both strands with universal forward and reverse primers by the dideoxy chain-termination method [9], using an automatic DNA sequencer by Mission Biotechnology Company. After identification of the correct sequence information, we used the two primers to screen the *P. monodon* haemocyte cDNA library following its publication [10]. Subsequently, PCR screening was conducted three times to choose a single phage plaque and in vivo excision. After in vivo excision, plating in LB-amp plates for plasmid cloning and maintenance in an *Escherichia coli* system (Stratagene, USA) was carried out. The nucleotide sequences were compared by searching the protein database (Blastx) with the NCBI translating Blast program. Sequences were translated into amino acid (aa) sequences and then analysed using GCG software. Multiple alignments with the corresponding amino acid sequences from other studies on chelonianin species were performed using the Pileup program, with default parameters. The percent of amino acids of *P. monodon* chelonianin identical with those of the other species of chelonianin was calculated using the NCBI protein–protein BLAST program.

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                                                    *
1    GGCACGAGGCAGCAGAGAACAGGACCTGATTCCCAAAATGGTGAACATCAA
                                                  M  V  N  I  K
52   GGCAGTTCTGATCGTGTGCGTTTTGGTGGCCGCGGTGGCTGTTTCTCCCGC
      A  V  L  I  V  C  V  L  V  A  A  V  A  V  S  P  A
103  CGATGCTGTTCCAACGAGACACAGTAAGCCTCGTCCTCAGCCTCTGCCCAG
      D  A  V  P  T  R  H  S  K  P  R  P  Q  P  L  P  R
154  GCCAGGAACGTGCCCAGATACGAGCGGCATCGTCACCACGTGCGAGGTGAC
      P  G  T  C  P  D  T  S  G  I  V  T  T  C  E  V  T
205  AGAACGCAACTGTCTCTCGGACAGCCAGTGCGGACCCGGCGAGAAGTGCTG
      E  R  N  C  L  S  D  S  Q  C  G  P  G  E  K  C  C
256  TTCGAGAGGCTGCGGGAGAGAGTGCCTGGCTGTGGTTCCTCCTTACGGAAG
                                 <-------------------- P1
      S  R  G  C  G  R  E  C  L  A  V  V  P  P  Y  G  S
307  TGGAAGGTAAAGGAGACAAATTTCAAGCTGCTAACGTCATCGCGGTTGGCT
      G  R  #
358  CAGAAACTTCTTGGGAAGATCATTAATGTGTGATGAGTCACTGCAAATAAA
409  ATTGAGTGGCTTATAAAAAAAAAAAAAAA
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Fig. 1. Nucleotide (nt) sequence of *Penaeus monodon* cDNA sequences and the predicted amino acids (aa) sequence of the gene. The nucleotides were numbered beginning with the first nucleotide at the 5′ end. (*) indicates a start codon; (#) indicates a stop codon. PCR primer 2 is located on phage vector arm. PCR primer 1 is located on arrow indication.

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