Electronic Journal of Biotechnology 32 (2018) 47–54

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Short communication

Molecular characterization and expression analysis of cathepsin C in Chinese giant salamander (*Andrias davidianus*) after *Aeromonas hydrophila* infection

Zisheng Wang [a], Panpan Hang [b], Qihuan Zhang [a], Qiaoqing Xu [b], Zhitao Qi [a,b,*]

[a] Jiangsu Key Laboratory of Biochemistry and Biotechnology of Marine Wetland, Yancheng Institute of Technology, Yancheng, Jiangsu, 224051, China
[b] College of Animal Sciences, Yangtze University, Jingzhou, Hubei Province, 434020, China

ARTICLE INFO

Article history:
Received 21 May 2017
Accepted 8 January 2018
Available online 2 February 2018

Keywords:
cDNA
CTSC
Dipeptidyl peptidase I
Gene expression
Hydrophila
Immune
Peptide
Sequence
Tissue

ABSTRACT

Background: Cathepsin C (CTSC) (dipeptidyl peptidase I, DPPI), is a member of the papain superfamily of cysteine proteases and involves in a variety of host reactions. However, the information of CTST in Chinese giant salamander (*Andrias davidianus*), an amphibian species with important evolutionary position and economic values, remained unclear.
Results: The full-length salamander CTSC cDNA contained a 96 bp of 5′-UTR, a 1392 bp of ORF encoding 463 amino acids, and a 95 bp of 3′-UTR. The salamander CTSC possessed several sequence features similar to other reported CTSCs such as a signal peptide, a propeptide and a mature peptide. The active site triad of Cys, His and Asn were also found existing in salamander CTSC. Salamander CTSC mRNA was constitutively expressed in all the examined tissues with significantly variant expression level. The highest expression of CTSC was in intestine, followed with stomach, spleen, lung and brain. Following *Aeromonas hydrophila* infection for 12 h, salamander CTSC was significantly up-regulated in several tissues including lung, spleen, brain, kidney, heart, stomach and skin.
Conclusion: CTSC plays roles in the immune response to bacterial infection, which provided valuable information for further studying the functions of CTSC in salamander.

1. Introduction

Cathepsin C (CTSC), also known as dipeptidyl peptidase I (DPPI), is a member of the papain superfamily of cysteine proteases [1], which is synthesized as an inactive precursor (zymogen), and is activated by a nonautocatalytic excision of an internal activation peptide within the N-terminal pro-peptide. The activated CTSC is consisted by "heavy" chain (231–394 amino acides) and "light chain" (395–463 amino acids) [2]. The activated CTSC may involve in a variety of host reactions, including intracellular protein degradation, cell growth, neuraminidase activation, and platelet factor XIII activation [3]. In addition, CTSC was found functioning as serine proteases in immune effector cells (mast cells, neutrophils, and lymphocytes) [4], and was essential for the interleukin (IL)-1-dependent sterile inflammatory response [5], indicating it may play roles in immune process.

Current studies on CTSC were mainly carried out in mammals, but were scarce in other animals. *Penaeus monodon* CTSC was up-regulated by lipopolysaccharide (LPS) [6], and *Fenneropenaeus chinensis* CTSC was up-regulated by *Vibrio anguillarum* and the white spot syndrome viruses (WSSVs) [7], implying that CTSC involves in immune defense against pathogens.

Chinese giant salamander is the largest extant urodela amphibian species. With the success of breeding of salamander, it has become an important economical aquatic species in central and western China. However, reports on the disease caused by pathogens in salamander, especially bacterial diseases, increased greatly in recent years. The bacterial diseases have become a hindrance to the healthy development of salamander industry [8]. Using immune strategies, e.g. developing vaccine for specific pathogen to treat or prevent the animal diseases has drawn great attention of researcher [9]. However, clearly understanding the animal immune system is a key condition for these strategies. Thus, in the present study, the salamander CTSC gene was cloned, and its expression in tissues of normal salamanders and *Aeromonas hydrophila* infected salamanders was analyzed,

* Corresponding author.
E-mail addresses: qizhitao@ycit.edu.cn, qzt_wh2002@163.com (Z. Qi).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2018.01.002

providing useful information for fully understanding the immune function of salamander CTSC in response to bacteria.

2. Materials and methods

2.1. Animal and bacterial infection

Normal Chinese giant salamanders (average body weight of 200 g) were purchased from a farm in Hubei province, China, and acclimated in aerated water tanks for one week before experiments. *A. hydrophila* (strain 4LNC209) was kindly provided by Professor Aihua Li (Institute of Hydrobiology, Chinese Academy of Sciences). Salamanders were randomly divided into two groups. One group was bacterial infected group, in which salamanders were injected intraperitoneally with *A. hydrophila* at a dose of 1.5×10^6 cfu/100 g body weight, and another group was control group, in which animals were injected with the same amount of PBS solution [8]. Ten tissues including liver, spleen, intestine, muscle, brain, stomach, kidney, lung, heart and skin from three animals in each group were sampled at 12 h post injection.

2.2. RNA extraction and reverse transcription

Total RNA of liver was isolated using Trizol reagent (Invitrogen, USA) according to manufacturer's instruction. Then, total RNA was reverse-transcribed using First Strand cDNA Synthesis Kit (Thermo Scientific, USA) based on manufacture's instruction.

2.3. Gene cloning of salamander CTSC

To obtain the salamander CTSC cDNA sequence, the liver transcriptome of salamander was searched by tBLASTn software using human CTSC (GenBank accession No. AAQ08887) as query bait. A sequence of 3215 bp in length was got, and this sequence was further analyzed using Translate at the ExPasy website (http://www.expasy.org/tools) to gain the potential open reading frame (ORF) and untranslated regions (UTRs). Then, specific primers were designed based on potential 5′-UTR and 3′-UTR, and polymerase chain reaction (PCR) amplification was done using liver cDNA as template. The PCR products were sequenced to confirm the correctness of sequences. Primers used in this study were listed in Table 1.

2.4. Sequence analysis

The amino acid (aa) of the nucleotide sequence was deduced using Translate at the ExPasy website (http://www.expasy.org/tools). The multiple alignments of aa sequences were done using Clustal O software (http://www.ebi.ac.uk/tools/msa/clustalo) and decorated with BoxShade software (http://www.ch.embnet.org/software/BOX_form.html). Protein sequence identity was calculated by MatGAT 2.02 software [10]. Isoelectric points and molecular weights were predicted with the ProtParam program (http://web.expasy.org/protparam). The signal peptide was predicted using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP). The protein motif was identified with PROSITE database (http://prosite.expasy.org/scanprosite/). Phylogenetic tree was constructed using Neighbor-Joining (N-J) method by Mega 7.0 software and the bootstrap was set as 10,000 to test the confidence of branch topology [11]. 3-D structure of CTSC was predicted by SWISS-MODEL workspace (https://swissmodel.expasy.org/) and the quality of structure was evaluated with PROCHECK program (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/).

Table 1
Primers for gene clone and expression analysis.

Primer	Sequence (5′-3′)	Application
adCTSC-F1	CATGTGACCTTCTGGAATCCAT	Gene clone
adCTSC-R1	GCATTGAAAATGTCAGAGTACATGG	Gene clone
adCTSC-F2	GTGGCCGCAACTCCCATATT	Gene expression
adCTSC-R2	GCCACAGGATGCTTGGTTTCG	Gene expression
adActin-F	CCACTGCTGCCTCCTCTT	Gene expression
adActin-R	GCAATGCCTGGGTACATG	Gene expression

2.5. Expression of CTSC in tissues of normal and A. hydrophila infected salamander

Expressions of CTSC in tissues of normal and *A. hydrophila* infected salamanders were detected using real-time quantitative PCR. Total RNA of each tissue mentioned above was extracted using Trizol reagent (Invitrogen, USA) according to manufacturer's instruction and then was reverse-transcribed using PrimeScript® reagent kit with gDNA eraser (TaKaRa, Japan) according to manufacturer's protocol. Real-time qPCR was performed using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) with SYBR Premix Ex Taq™ (TaKaRa, Japan) according to our previous studies [12,13]. Briefly, the cDNA fragments of CTSC and *β-actin* were amplified by RT-PCR. The amplicons in same equal molar amounts were serially ten-fold diluted and run along with the cDNA test samples on the same 96-well PCR plate as quantitative reference. The expression of CTSC in each tissue of normal salamander was normalized to the expression level of β-actin and expressed ad arbitrary units [12,13]. The expression change of CTSC in tissues of *A. hydrophila* infected salamanders was expressed as fold change according to our previous studies [12,13].

3. Results

3.1. Sequence analysis of salamander CTSC

The salamander CTSC cDNA was 1583 bp in length, with a 96 bp of 5′-UTR, a 1392 bp of ORF encoding 463 amino acids, and a 95 bp of 3′-UTR (Fig. 1). The predicted molecular mass and theoretical isoelectric point of salamander CTSC was 52.55 kDa and 5.8, respectively. The deduced amino acid of salamander CTSC shared high sequence identity with that of human (69.8%), mouse (69.0%), turtle (74.0%) and zebrafish (66.2%). Sequence alignment revealed that there existed a 23 aa of signal peptide, a 207 aa of long propeptide region (position of 24–230 aa), and a 233 aa of mature peptide region (position of 231–463 aa) in salamander CTSC (Fig. 2). The mature protein was consisted of a 164 aa of heave chain (position 231–394 aa) and a 69 aa of light chain (position of 395–463 aa), among which contained three conserved catalytic active sites (Cys^{258}, His^{405} and Asn^{427}) (Fig. 2). In addition, three potential N-glycosylation sites were found in salamander CTSC, with two in the proregion (at position 28 and 55) and one in the mature peptide (at position 276).

Further, phylogenetic tree analysis showed that vertebrates' CTSC were clustered into one clade, which was separated clearly from clade of CTSK, CTSS, CTSL, CTSH, CTSA, CTSD and CTSB. In the CTSC clade, salamander CTSC showed close relationship with turtle CTSC, which was in line with the results of sequence identity analysis. In each clade of the phylogenetic tree, the position of each sequence was in agreement with that of traditional taxonomy (Fig. 3).

3.2. Gene synteny analysis of vertebrates' CTSC loci

The gene synteny of vertebrates' CTSC loci was analyzed using Genomicus (v85.01) software. Results showed that the CTSC loci were highly conserved in vertebrates. The conserved genes near CTSC were almost found in all vertebrates, such as RAB138, GRM5, and TRY. In addition, the transcriptional directions of genes in this loci were compatible (Fig. 4). However, the gene linked with CTSC was different in lamprey (Fig. 4).