Gene 508 (2012) 241–249

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Molecular functions of chaperonin gene, containing tailless complex polypeptide 1 from *Macrobrachium rosenbergii*

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ARTICLE INFO

Article history:
Accepted 30 July 2012
Available online 8 August 2012

Keywords:
Chaperonin
Prawn
Virus
Chaperone activity
ATPase activity

ABSTRACT

Chaperonin (*Mr*Chap) was identified from a constructed transcriptome dataset of freshwater prawn *Macrobrachium rosenbergii*. The *Mr*Chap peptide contains a long chaperone super family domain between 11 and 525. Three chaperone tailless complex polypeptide (TCP-1) signatures are present in the *Mr*Chap peptide sequence at 36–48, 57–73 and 85–93. The gene expressions of *Mr*Chap in both healthy *M. rosenbergii* and those infected with infectious hypodermal and hematopoietic necrosis virus (IHHNV) were examined using qRT-PCR. To understand its biological activity, the recombinant *Mr*Chap gene was constructed and expressed in *Escherichia coli* BL21 (DE3). The results of ATPase assay showed that the recombinant *Mr*Chap protein exhibited apparent ATPase activity. Chaperone activity assay showed that the recombinant *Mr*Chap protein is an active chaperone. These results suggest that *Mr*Chap is potentially involved in the immune responses against viral infection in *M. rosenbergii*. These findings indicate that the recombinant *Mr*Chap protein may be used in immunotherapeutic approaches.

1. Introduction

Chaperonins are a class of proteins which ease the folding and association of nascent polypeptide chains in bacteria, fungi, plants and animals. Folding is an essential step for assembling the polypeptides and this may avoid dissociation by hydrophobic residue during its native states (Gupta et al., 2006). Thus chaperonin emphasizes its vital presence in cellular mechanism as it directs the sequester protein folding and ensures dynamic equilibrium of the protein level in the system. Chaperonin function was presupposed from the genetic studies with the *Escherichia coli*/bacteriophage λ (Friedman et al., 1984). The identification of these proteins has increased the understanding of protein folding *in vivo* (Gupta et al., 2006). So far, two chaperonins have been distinguished and were classified as groups I (GroEL/GroES) and II (thermosome).

The group I chaperonin are tetradecameric complexes composed of one or two kinds of subunits, named after the bacterial genes that it was identified, chaperonin proteins GroES (homolog in *E. coli* is GroE small; also known as chaperonin 10) with molecular mass of about 10 kDa and GroEL (homolog in *E. coli* is GroE large; also known as chaperonin 60) with molecular mass of 60 kDa. The formation of this group I chaperonin is accelerated by several external factors including bacteriophage λ infection, heat, ultraviolet light (UV) radiation and chemical reagents. The group II chaperonin is heterogeneous in nature, mostly available in archaebacteria and eurkaryote cytosol (known as TriC or CCT; TCP1-ring complex or chaperonin containing TCP1). There are about nine kinds of subunits that reside in the eukaryotic cytosolic chaperonin, where its rotational symmetry is up to 8 or 9 fold (Kubota et al., 1995). Both the GroEL and GroES exist in a heptamer structure with each having double and single ring respectively. The middle of the double ring heptamer of GroEL is filled with chaperonin and this complex binds to the GroES single heptamer ring.

In a well understood mechanism of protein folding involving chaperonin GroEL and GroES, the newly synthesized polypeptides are arbitrated to folding by GroEL in an ATP dependant reaction (Hartl, 1996). In conjunction with that, the GroEL undergoes structural changes to prevent other protein binding to reduce competition for

Abbreviations: *Mr*Chap, *Macrobrachium rosenbergii* chaperonin; TCP-1, tailless complex polypeptide; IHHNV, infectious hypodermal and hematopoietic necrosis virus; qRT-PCR, quantitative real time polymerase chain reaction; ATP, adenine tri-phosphate; GroES, homolog in *E. coli* is GroE small; GroEL, homolog in *E. coli* is GroE large; CCT, TCP1-ring complex or chaperonin containing TCP1; HSPs, heat shock proteins; IPTG, isopropyl-β-thiogalactopyranoside; MBP, mannose binding protein; ORF, open reading frame; UTR, untranslated region.

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http://dx.doi.org/10.1016/j.gene.2012.07.050

Table 1
ScanProsite motif analysis of *Mr*Chap amino acid.

Details of domain and motifs (nos.)	AA position
Domain:	
Chaperone super family (1)	11–525
Signature motifs:	
Chaperone tailless complex polypeptide-1 (TCP-1)	36–38
TCP-2	57–73
TCP-3	85–93
Non-organellar eukaryotic consensus motif	310–318
Glycine-methionine motif	527–544
Common motifs:	
N-myristoylation site (5)	11–16, 159–164, 327–332, 362–367 and 533–538
Protein kinase C phosphorylation site (6)	123–125, 139–141, 321–323, 384–386, 519–521 and 522–524
Casein kinase II phosphorylation site (7)	77–80, 152–155, 203–206, 332–335, 387–390, 515–518 and 525–528
N-glycosylation site (2)	337–340 and 487–490
cAMP and cGMP dependent protein kinase phosphorylation site (1)	125–128

the active ring site. Besides this, the GroES attachment to GroEL also eases the protein folding mechanism.

Molecular chaperonin plays a vital role in the immune response of variety of organisms, where they are also termed as heat shock proteins (HSPs) as it assists in the formation of protein in stress condition. As in the study of chaperonin in immunity of shrimp, the first cloning and characterization of heat shock cognate 70 was reported by Lo et al. (2004) and it was conserved in both vertebrates and invertebrates. There has been a few reports available on the findings of this molecular chaperonin in other species including zhikong scallop *Chlamys farreri* (Gao et al., 2007), bay scallop *Argopecten irradiant* (Gao et al., 2008), zebra fish *Danio rerio* (Matsuda and Mishina, 2004) and migratory locust *Locusta migratoria* (Qin et al., 2003).

Macrobracium rosenbergii, or giant freshwater prawn has a high economical value in the aquaculture industry compared to other freshwater cultured crustaceans in all over the World. However the shrimp aquaculture industry has been facing a major crisis due to various viral diseases. More than 20 viruses, which include infectious hypodermal and hematopoietic necrosis virus (IHHNV) have been identified in prawns (Arockiaraj et al., 2012a). Thus the research on immune mechanism of freshwater prawns especially *M. rosenbergii* has to be improved in order to sustain the productivity of these economically important species.

So far, only a limited number of reports have been published on molecular cloning, characterization and gene expression of chaperonin from crustacean, but as per our knowledge, there is no report available on chaperonin from *M. rosenbergii*. To gather the knowledge in the characterization of *M. rosenbergii* chaperonin (designated as *Mr*Chap) and its role in *M. rosenbergii*, a full length cDNA of *Mr*Chap was identified from the transcriptome of *M. rosenbergii* unigenes obtained by Illumina's Solexa sequencing technology. The transcriptional differentiation of *Mr*Chap mRNA has been analyzed using IHHNV challenge. Furthermore, over expression and purification of recombinant *Mr*Chap protein were conducted using *E. coli* BL21 (DE3) bacterial expression system and analyzed their functional properties.

2. Materials and methods

2.1. Prawns

Healthy prawns (average body weight of 10 g) were obtained from the Bandar Sri Sendayan Aquaculture Farm in Negeri Sembilan, Malaysia. Prawns were maintained in flat-bottomed glass tanks (300 L) with aerated and filtered freshwater at 28 ± 1 °C in the laboratory. All prawns were acclimatized for 1 week before being challenged to IHHNV. A maximum of 15 prawns per tank were maintained during the experiment.

2.2. M. rosenbergii chaperonin

A full length *Mr*Chap gene was identified from the *M. rosenbergii* transcriptome unigenes obtained by Illumina's Solexa sequencing technology. In brief, unigenes obtained from the assembly of the Illumina Solexa short reads of the RNA sequencing of the muscle, gills and hepatopancreas transcriptomes of *M. rosenbergii* were mined for sequences which had been identified as chaperonin gene through BLAST homology search against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast).

2.3. Chaperonin sequence analysis

The full-length *Mr*Chap sequence was compared with other sequences available in NCBI database and the similarities were analyzed. The open reading frame (ORF) and amino acid sequence of *Mr*Chap was obtained by using DNAssist 2.2. Characteristic domains or motifs were identified using the PROSITE profile database (http://prosite.expasy.org/scanprosite/). The N-terminal transmembrane sequence was determined by DAS transmembrane prediction program (http://www.sbc.su.se/~miklos/DAS). Signal peptide analysis was done using the SignalP (http://www.cbs.dtu.dk). Pair-wise and multiple sequence alignment were analyzed using the ClustalW version 2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The phylogenetic relationship of *Mr*Chap was determined using the Neighbor-Joining (NJ) method and PHYLIP (3.69). The presumed tertiary structures were established for *Mr*Chap using the SWISS-MODEL prediction algorithm (http://swissmodel. expasy.org/).

2.4. Gene expression of MrChap after IHHNV infection

For IHHNV induced gene expression analysis, the prawns were injected with IHHNV, as described in our earlier report (Arockiaraj et al., 2011). IHHNV infected prawn tail tissue, tested positive by nested PCR was homogenized in sterile 2% NaCl (1:10, w/v) solution and centrifuged in a tabletop centrifuge at 5000 rpm for 5 min at 4 °C. The supernatant was filtered through 0.45 μm filter and used for injecting (100 μl per 10 g prawn) the animals. Samples were collected before (0 h), and after injection (3, 6, 12, 24 and 48 h) and were immediately snap-frozen in liquid nitrogen and stored at −80 °C until total RNA was isolated. Using a sterilized syringe, the hemolymph (0.2–0.5 ml per prawn) was collected from the prawn heart and immediately centrifuged at 3000 × *g* for 10 min at 4 °C to allow hemocyte collection for total RNA extraction. Tissue homogenate prepared from healthy tail muscle served as control. All samples were analyzed in

Fig. 1. Multiple sequence alignments of *Mr*Chap with six other homologous sequences. Chaperonin 1 from brine shrimp *Artemia franciscana* (AAL27405), chaperonin 7 from zebrafish *Danio rerio* (AAI63876), pipid frog *Xenopus tropicalis* (AAH89710), chaperonin from yellow fever mosquito *Aedes aegypti* (EAT48473), castor oil plant *Ricinus communis* (EEF33578) and human *Homo sapiens* (AAC96011) are shown. Chaperone tailless complex polypeptide (TCP-1) signature 1 (36–48), 2 (57–73) and 3 (85–93) are highlighted in green, pink and blue color, respectively. The potential nonorganellar eukaryotic consensus motif is boxed. Glycine–methionine motif is highlighted in yellow color. Asterisk marks indicate identical amino acids and numbers to the right indicate the amino acid position of chaperonin in the corresponding species. Conserved substitutions are indicated by (:) and semi-conserved substitutions are indicated by (.). Deletions are indicated by dashes. GenBank accession numbers for the amino acid sequences are given in the parentheses.