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First *hsp70* from two hydrothermal vent shrimps, *Mirocaris fortunata* and *Rimicaris exoculata*: Characterization and sequence analysis

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Abstract

The vent shrimps, *Mirocaris fortunata* and *Rimicaris exoculata*, live in a highly fluctuating thermal environment and undergo frequent temperature bursts. As a first step in the investigation of the response to heat stress, this work aimed to characterize stress proteins in these two species. Complementary deoxyribonucleic acid (cDNA) clones encoding a 70-kDa heat shock protein (HSP) were isolated and characterized from *M. fortunata* and *R. exoculata*. The cDNA clones were of 2055 and 1941 base pairs in length, and contained a 2018-bp complete open reading frame (ORF) and a 1785-bp partial coding sequence, respectively. The amino acid sequences corresponding to these ORF are 645 residues in length for *M. fortunata* and 595 for *R. exoculata*, and were clearly characterized as members of the HSP70 family. The C-terminal extremity would identify *R. exoculata* sequence as a cytoplasm HSP70. The relationships between the crustacean HSP70 sequences were examined by two phylogenetic methods, i.e. Maximum Likelihood and Bayesian methods. The resulting trees suggested that *M. fortunata* sequence may correspond to constitutively expressed HSP70, named HSC70, whereas *R. exoculata* sequence may correspond to an inducible form of HSP70. The HSP70 sequences from the hydrothermal shrimps proved to be very similar to the other homologous shrimp sequences, except for the presence of an insertion of unknown function in the ATPase domain of *R. exoculata* sequence.

Keywords: Heat shock protein; Stress protein; Chaperone; Heat stress; Crustacea; Deep-sea vent

1. Introduction

Organisms are able to deal with the insults of thermal fluctuations in the ambient environment through highly conserved cellular defense mechanisms. When cells encounter heat stress, they provoke active responses in order to retune their internal milieu. The most well characterized heat shock response is the induction of a highly conserved set of polypeptides termed heat shock proteins (HSP) (Parsell and Lindquist, 1993). Because many different stressors trigger increases in the expression of HSP (Lindquist, 1986), these proteins are also referred to as stress proteins. Under conditions of stress, HSP are known to provide an essential action, by preventing aggregation and assisting refolding of misfolded proteins. But several members of the HSP family also play an essential role in un-stressed cells, where they act as molecular chaperones to assist a wide range of folding processes (see Mayer and Bukau, 2005 for review). Stress proteins are thus arbitrarily designated as constitutively expressed (cognate stress proteins) or stress-inducible, although constitutively expressed HSPs can be up-regulated under stress conditions (Feder and Hofmann, 1999). HSP range in size from 27 to 110 kDa and can be divided into five groups based on both size and function: small

Abbreviations: cDNA, DNA complementary to RNA; hsp, heat shock protein; kDa, kilodaltons; mRNA, messenger RNA; nt, nucleotides; ORF, open reading frame; RNase, ribonuclease; RT, reverse transcriptase; PCR, polymerase chain reaction; BP, Bootstrap percentages; UTR, UnTranslated Region.

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HSP (20–40 kDa), HSP60, HSP70, HSP90, HSP100 (Parsell and Lindquist, 1993; Moseley, 1997; Borkan and Gullans, 2002). Among the different families of HSP, the 70 kDa family (HSP70) is the most conserved and has been most extensively studied. Well-recognized members of the HSP70 multigenic family are the stress-inducible HSP70 and the constitutively expressed HSC70 proteins, present in all organisms studied to date (Lindquist and Craig, 1988). HSP70 proteins have been shown to play a critical role in cell survival and thermotolerance in response to stresses (Parsell and Lindquist, 1993).

Rimicaris exoculata (Williams and Rona, 1986) and *Mirocaris fortunata* (Martin and Christiansen, 1995; Komai and Segonzac, 2003), two species of caridean shrimps belonging to the family Alvinocarididae, dominate the vagile megafauna at Mid-Atlantic Ridge hydrothermal vent sites (Desbruyères et al., 2001). These shrimps live in a highly fluctuating thermal environment and often undergo temperature bursts of up to about 20 °C (Desbruyères et al., 2001). *R. exoculata* swarms around hydrothermal black smoker chimneys, at the hot end of the hydrothermal biotope, and damages (scalded cuticle) caused by heat exposure were observed on collected specimens (Van Dover et al., 1988; Gebruk et al., 1993; Gebruk et al., 2000). Although closely related to *R. exoculata*, *M. fortunata* is nevertheless more broadly distributed across the vent-fluid influence gradient, and is supposed to be a less thermotolerant species (Desbruyères et al., 2001; Shillito et al., 2006). These two species are obviously of interest for studying the response to heat stress. We previously determined the critical thermal maximum of these two shrimp species below 40 °C (Ravaux et al., 2003; Shillito et al., 2006). The temperature resistance of the vent shrimps appeared to be very similar to those of other non-vent caridean shrimps. This raises the question of the biological processes that allow the vent shrimps to face severe and frequent temperature bursts. As a first step in the investigation of the response to heat stress, the aim of this work was to identify and characterize stress proteins belonging to the HSP70 family in *M. fortunata* and *R. exoculata*. Until now, there was no data on HSP available for hydrothermal vent metazoans, except for an immunodetection of HSP70 proteins in *R. exoculata* (Ravaux et al., 2003).

We here provide the first data on stress proteins originating from hydrothermal metazoans. Two cDNAs (2055 and 1941 nt-long) encoding *hsp70* in the hydrothermal shrimps *M. fortunata* and *R. exoculata* were identified and characterized. To obtain information on these *hsp70* related to their function in the cell and possibly to their adaptation to hydrothermal environment, we studied their primary structure, and compared it to available *hsp70* sequences for other crustaceans (see Table 1). We examined the relationships between *hsp70* by two different phylogenetic methods, i.e. Maximum Likelihood and Bayesian methods.

2. Materials and methods

2.1. Biological material

R. exoculata and *M. fortunata* specimens were collected during the 'ATOS' cruise (R/V *Atalante*, ROV *Victor6000*, June 2001), at the Rainbow vent site (36°14.0′ N, Mid-Atlantic Ridge, 2300 m depth). Animals were sampled with a suction device operated by the submersible's hydraulic arm, and stored inside insulated Perspex cylinders, until transferred to the ship. Upon recovery, the temperature of the water inside the cylinders ranged from 10 to 15 °C. Specimens of *M. fortunata* were frozen as soon as they arrived on board and stored in liquid nitrogen. Specimens of *R. exoculata* were placed in vessels at *in situ* pressure, and heat exposed to a temperature of 25 °C (for details see Ravaux et al., 2003). The shrimps experimented were frozen in liquid nitrogen on board until further analyses at the lab.

2.2. Electrophoresis and immunodetection of HSP70

Samples of shrimp abdomens without their cuticle were ground in liquid nitrogen, and the powder was homogenized in 1 ml of extraction buffer [50 mM Tris/HCl, pH 7.4:protease inhibitor cocktail (Sigma, St Quentin Fallavier, France) 1:3 (v/v)]. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C, and the extracted proteins were quantified in the supernatant with the Bio-Rad Protein Assay (Bio-Rad, Marnes-la-Coquette, France) using bovine serum albumin (Sigma) as standard. The electrophoretic separation of the proteins and western blot detection of HSP70 were done as previously described by Ravaux et al. (2003).

2.3. RNA extraction and reverse transcriptase

Tissues from shrimp abdomens, without their cuticle, were ground in liquid nitrogen. The powder was homogenized in Trizol Reagent (Invitrogen) and total RNA was isolated according to the manufacturer's protocol, and quantified by spectrophotometry and electrophoresis in a 1.2% agarose gel under denaturing conditions.

Single-stranded cDNAs were synthesized from 3 μg of shrimp abdomen total RNA using an oligo(dT)-anchor primer and MMLV reverse transcriptase (200 units, Promega). The reaction mixture contained dNTP mix (Promega), RNasin (Promega), oligo dT-anchor primer (supplied in the 3′–5′RACE kit from Roche) or a specific HSP primer (see below 5′RACE), sterile water and RNA template dissolved to a final volume of 25 μL. This solution was heated at 65 °C for 5 min before addition of the enzyme and incubation for 1 h at 37 °C and 15 min at 75 °C.

2.4. PCR amplifications

The cDNA fragments encoding the shrimp HSP70 were amplified by two rounds of PCR amplification. Oligonucleotide primers for the *hsp70* were designed from multiple alignment of homologous sequences of crustaceans (*Penaeus monodon* AAQ05768, *Artemia franciscana* AAL27404), molluscs (*Crassostrea gigas* CAC83009, *Crassostrea ariakensis* AAO41703, *Ostrea edulis* CAC83010, *Biomphalaria glabrata* AAB95297), and an insect (*Manduca sexta* AAO65964). In the first PCR, amplification was primed by pairs of degenerated oligonucleotides (HSP1 and HSP3, see Fig. 1 and Table 2) and the reactions were performed in a 20 μl volume containing 1 μl of cDNA template, 1.5 mM $MgCl_2$,