Comparative sequence analysis of Hsp70 gene from Mytilus edulis desolationis and Aulacomya ater of the Kerguelen Islands

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Introduction

Circumpolar studies have provided strong evidence of the vulnerability of marine ecosystems in response to anthropogenic activities. Such ecological disturbances are particularly impactful on biodiversity, which helps protect ecosystems from extreme conditions. It is thus imperative to develop biomarkers for long term monitoring of changes in marine biodiversity and to better assess comparative responses of different species. Because of their wide geographic distribution and their feeding and filter-feeding nature, mussels, such as Mytilus edulis and its closely related species, have been commonly used as sentinel species in ecotoxicological monitoring programs around the globe.1,3 Extensive research has been performed on their physiology and their genetic content in order to develop sensitive and specific biomarkers. This is especially true for M. edulis, which has been extensively characterized at the cellular and molecular levels, not only because of their ecological importance, but also because of their economical impact.1,4 However, because of their distinctive anatomy and physiology, it is logical to believe that other mussel species will respond differently to climate change and exposure to pollutants.7 Our recent comparative study between Mytilus desolationis (M. desolationis) and Aulacomya ater (A. ater) supports this hypothesis.4 A better knowledge of A. ater could thus provide a new and complementary tool for monitoring global climate changes in marine ecosystems, most notably in the Southern hemisphere, which is particularly sensitive to climate change. Because of its strategically geographical position, the Kerguelen archipelago is considered an important site to investigate the effects of global change on marine ecosystems.7 Although M. desolationis is normally the dominant species in most mussel beds in the Kerguelen archipelago, we found that some mussel beds, such as one found at tide depth at Port-aux-Français, is largely dominated by A. ater.8 Whether such diversity is permanently maintained or will change following environmental perturbation will depend on the ability of mussel species to respond to environmental stress. Because heat shock proteins (Hsps) play an important role of protection and maintenance of many vital cellular functions in response to thermal and toxic stress, they are commonly used as stress biomarkers.9,10 Their structural features are highly conserved among eukaryotes and prokaryotes, especially in the case of hsp70, a widely used biomarker to monitor the impact of environmental factors on various animal species, including mussels.12,15 Unfortunately, while a considerable amount of data exists on blue mussel stress response genes, our knowledge on A. ater remains fragmentary. In the present work, we report a detailed comparative analysis of hsp70 stress response gene from Mytilus desolationis (M. desolationis) and Aulacomya ater (A. ater).

Materials and Methods

Collection of specimens

Adult specimens (55–70 mm length) of blue mussels, M. edulis desolationis, and ribbed mussels, A. ater were collected on the intertidal rocky shore of Port-aux-Français (Kerguelen Islands, France). Mussels were immediately transferred to the marine laboratory of Port-aux-Français and placed in a temperature-controlled (12°C) aerated aquarium containing filtered recirculating seawater (Kerguelen Islands, France). Mussels were dissected, tissues frozen in liquid nitrogen, and stored at –80°C until further analysis.

RT-PCR and sequencing

Tissues (gills) were homogenized by sonication and total cellular RNA was isolated using the TRIzol reagent (Life Technologies) according to the manufacturer’s instructions. Ultraviolet (UV) absorbance was used to assess the quality and the concentrations of RNA. First-strand cDNA was prepared from cellular RNA using the reverse transcriptase Omniscript (QIAGEN, Mississauga, ON, Canada). cDNAs were amplified using specific primers (Table 1) with the following conditions: 94°C for 3 min, followed by 30 to 35 cycles of the following: 94°C for 40 sec, 60°C for 40 sec (unless otherwise indicated), and 72°C for 40 sec, followed by a final extension step at 72°C for 10 min. PCR was performed in a thermal cycler (MJ Research, Watertown, MA). The amplified products were analyzed by electrophoresis using 1.5% (w/v) agarose gels, SYBR Safe (Life Technologies) DNA gel staining and UV illumination. Sequencing of the amplicons was performed by the Genome Quebec sequencing platform. Structure figures were prepared in PyMOL (The PyMOL Molecular Graphics System).

Results

Stress-induced ATP-bound hsp70 binds to newly synthesize unfolded or partially folded proteins via interaction with hydrophobic peptide segments and prevents their aggregation into nonfunctional structure. Once the entire protein is synthesized, it binds to nucleotide exchange factors (NEFs), such as BAG, thereby inducing the release of ADP and binding of fresh ATP, opening the binding pocket (reviewed in8). Hsp70 binds ATP via its N-terminal ATPase domain (NBD), which consists of two lobes with a deep cleft between them, at the bottom of which nucleotide (ATP and ADP)
Table 1. List of oligonucleotides used for amplification of hsp70 of *A. ater* and *M. desolatissis*.

| Name of primers       | Primer sequences                      | Temperature(°C) | Position | Amplicon Size (bp) |
|-----------------------|---------------------------------------|-----------------|----------|-------------------|
| **M. desolatissis**   |                                       |                 |          |                   |
| Hsp70gallo467F        | 5’CTGCACCTGGCTATAGCTGAC3’             | 62°C            | 606-625  | 467               |
| Hsp70gallo467R        | 5’CATGGACAGCAGCCCTTTG3’               |                 | 1050-1070|                   |
| Hsp70gallo488F        | 5’AGCTGGGAGCTGAGCTGAC3’               | 62°C            | 1051-1070| 448               |
| Hsp70gallo351F        | 5’TTCACATCGGCAACGAC3’                 | 60°C            | 1667-1687| 351               |
| Hsp70gallo351R        | 5’GGTGGTGGCTACGAC3’                   |                 | 1667-1687|                   |
| Hsp70gallo488F        | 5’CTGGTGCTACGACGAC3’                  | 60°C            | 1200-1219| 487               |
| Hsp70gallo487F        | 5’TCCACATCGGCAACGAC3’                 |                 | 1667-1686|                   |
| Hsp70gallo487R        | 5’AGCTGGGAGCTGAGCTGAC3’               |                 | 843-864  | 829               |
| **A. ater**           |                                       |                 |          |                   |
| Hsp70ater2265F        | 5’ATCGACCGAAAGCCCGCCCTTTG33’         | 61°C            | 278-299  | 205               |
| Hsp70ater353R         | 5’ATGCTGGGACGGGACGGCCCTTTG33’        |                 | 482-461  |                   |
| Hsp70ater2265F        | 5’ATCGACCGAAAGCCCGCCCTTTG33’         | 60°C            | 1668-1708| 265               |
| Hsp70ater366R         | 5’ATGCTGGGACGGGACGGCCCTTTG33’        |                 | 1590-1531|                   |
| Hsp70ater220bF        | 5’ATCGACCGAAAGCCCGCCCTTTG33’         | 59°C            | 1681-1703| 220               |
| Hsp70ater353R         | 5’ATGCTGGGACGGGACGGCCCTTTG33’        |                 | 1590-1531|                   |
| Hsp70ater220bFF       | 5’ATCGACCGAAAGCCCGCCCTTTG33’         | 59°C            | 1667-1686| 366               |
| Hsp70ater366R         | 5’ATGCTGGGACGGGACGGCCCTTTG33’        |                 | 2022-2011|                   |
| Hsp70ater353R         | 5’ATGCTGGGACGGGACGGCCCTTTG33’        | 61°C            | 1667-1686| 353               |

*Figure 1. Simplified cladogram showing relationships between mussel species based upon Hsp70 sequence. The cladogram was constructed using the CLUSTAL W (1.83) multiple sequence T-coffee alignment software (http://www.ebi.ac.uk/Tools/msa/tcoffee/).*
have been studied in details for many years, molecular characterization of A. ater genes remains anecdotic at best. Our results showed that hsp70 from A. ater is significantly different from that of M. desolationis. Such difference can be exploited as a tool to discriminate between M. desolationis and A. ater samples at the molecular level. The technique is simple to perform and can be implemented in all settings where PCR is available. The molecular characterization of hsp70 in both species provides a new tool to better assess in the future the ability of both species to environmental stress. Our recent study did indeed show that both species respond differently to cal- cium-induced apoptosis. Although hsp70 is among the most highly conserved gene during evolution, at the protein level, our results also showed that hsp70 of A. ater harbors more than 30 amino acid substitutions when compared to hsp70 of blue mussels. Because the substrate specificity of chaperones like hsp70 is dictated by minor changes in the primary amino acid sequence, these changes may indicate fundamental differences between hsp70 activity in M. desolationis and A. ater. Whether such differences may alter their ability to respond to environmental stress is an interesting possibility given the progressive disappearance of A. ater from many marine habitats. The development of novel molecular stress biomarkers of A. ater could provide a new and complementary tool for monitoring global climate changes in marine ecosystems in the Southern hemispheres, which is particularly sensitive to climate change.

In conclusion, we report the molecular characterization of hsp70 in A. ater and highlight major differences at the nucleic acid and protein levels with M. desolationis. This information will be useful for the development of new molecular tools to monitor the effect of environmental stress on both mussel populations, which are well known for their ability to co-exist in the same marine habitat. This information will also be useful for learning the phylogenetic relationships between both mussel species and with other A. ater populations present in various marine ecosystems in the Southern hemisphere.

**Discussion**

Because of cellular abundance and high degree of conservation, hsp70 is perhaps the best-studied stress biomarker. In the present work, we provide the first molecular characterization of hsp70 in A. ater. In contrast to other mussel species, such as M. edulis and *M. galloprovincialis*, which have been studied in details for many years, molecular characterization of A. ater genes remains anecdotic at best.

**Table 2. Percentages of identity of hsp70 at the amino acid level between species.**

| Species          | M. desolationis | A. ater | M. galloprovincialis |
|------------------|-----------------|--------|---------------------|
| M. desolationis  | 100%            | 95%    | 99%                 |
| M. galloprovincialis | 99%            | 95%    | 100%                |
| M. coruscus      | 99%             | 94%    | 99%                 |
| S. virgatus      | 93%             | 93%    | 94%                 |
| A. ater          | 95%             | 100%   | 95%                 |
| P. viridis       | 94%             | 93%    | 94%                 |
| C. fluminae      | 91%             | 90%    | 91%                 |
| H. sapiens       | 90%             | 88%    | 90%                 |
| M. musculus      | 90%             | 88%    | 90%                 |
| C. gigas         | 91%             | 90%    | 91%                 |
| C. elegans       | 68%             | 67%    | 70%                 |
| D. melanogaster  | 74%             | 73%    | 76%                 |

**Table 3. Amino acid substitutions in hsp70 of M. desolationis and A. ater as compared to hsp70 of M. galloprovincialis.**

| Substitution  | M. galloprovincialis → A. ater | M. galloprovincialis → M. desolationis |
|---------------|--------------------------------|---------------------------------------|
| Ala3 → Ser4  | Lys15 → Glu21                  | Glu26 → Ser21                         |
| Thr2 → Ser4  | Ile41 → Val50                  | Val112 → Thr112                       |
| Pro→ deleted | Gly215 → Asp234                | Thr112 → Ser112                       |
| Ile3 → Val   | Ala21 → Ser21                  | Gly234 → Arg234                       |
| Val15 → Ser21| Ala30 → Asp30                  | Val15 → Gly214                       |
| Asp31 → Glu74| Ala111 → Gly111                | Ala111 → Glu111                      |
| Thr12 → Asp31| Glu114 → Asn114                | Thr12 → Asp31                        |
| Asn5 → Ser10 | Lys92 → Arg92                  | Asn5 → Ser92                         |
| Ser23 → Gly50| Gln102 → Ser102                | Ser23 → Gly102                       |
| Val104 → Ile104 | Gly255 → Glu255              | Val104 → Ile104                     |
| Thr112 → Lys112 | Gly265 → Ala214              | Thr112 → Lys112                      |
| Ile1230 → Val | Ser288 → Asp285               | Val2120 → Ile2120                    |
| Leu133 → Gln133 | Gly271 → Val271              | Leu133 → Gln133                      |
| Val110 → Ile100 | Gly271 → Val271              | Val110 → Ile100                      |
| Ser115 → Ala145 | Asp277 → Gly277           | Ser115 → Ala145                      |
| Gly130 → Arg156 | Ile273 → Val273             | Gly130 → Arg156                      |
| → Ser320 → Asn131 → Ala156 | Gly142 → Ser142         | → Ser320 → Asn131 → Ala156          |
Our results showed that hsp70 from A. ater is significantly different from that of M. desolationsis. Such difference can be exploited as a tool to discriminate between M. desolationsis and A. ater samples at the molecular level. The technique is simple to perform and can be implemented in all settings where PCR is available. Most importantly, the molecular characterization of hsp70 in both species provide a new tool to better assess the ability of both species to environmental stress. Our recent study did indeed show that both species respond differently to cadmium-induced apoptosis8. Although hsp70 is among the most highly conserved gene during evolution, at the protein level, our results also showed that hsp70 of A. ater harbors more than 30 amino acid substitutions when compared to hsp70 of blue mussels. Because the substrate specificity of chaperones like hsp70 is dictated by minor changes in the primary amino acid sequence19, these changes may indicate fundamental differences between hsp70 activity in M. desolationsis and A. ater. Whether such differences may alter their ability to respond to environmental stress is an interesting possibility given the progressive disappearance of A. ater from many marine habitats2. The development of novel molecular stress biomarkers of A. ater could provide a new and complementary tool for monitoring global climate changes in marine ecosystems in the Southern hemisphere, which is particularly sensitive to climate change.

Conclusions

In conclusion, we report the molecular characterization of hsp70 in A. ater and highlight major differences at the nucleic acid and protein levels with M. desolationsis. This information will be useful for the development of new molecular tools to monitor the effect of environmental stress on both mussel populations, which are well known for their ability to co-exist in the same marine habitat. This information will also be useful for learning the phylogenetic relationships between both mussel species and with other A. ater populations present in various marine ecosystems in the Southern hemisphere.

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