Immunobiology 219 (2014) 850–858

Contents lists available at ScienceDirect

Immunobiology

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

Study on the immune response to recombinant Hsp70 protein from *Megalobrama amblycephala*

Nan Chen[a,b], Xiao-Ling Wan[a], Chun-Xiao Huang[a,b], Wei-Min Wang[a,b], Hong Liu[a,b], Huan-Ling Wang[a,b,*]

[a] *Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Fishery, Huazhong Agricultural University, 430070 Wuhan, PR China*
[b] *Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, 430070 Wuhan, PR China*

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 3 April 2014
Accepted 15 July 2014
Available online 21 July 2014

Keywords:
Megalobrama amblycephala
Recombinant Hsp70
Immune response

ABSTRACT

The expression of heat shock protein 70 (Hsp70) is induced in response to many factors including high temperature, infection, metal pollutants and toxic chemicals. In this study, *Megalobrama amblycephala HSP70* promoter was cloned, and characteristic heat shock elements (HSEs) were identified in the promoter region. The recombinant *M. amblycephala* Hsp70 protein (rMaHsp70) was expressed and purified from *Escherichia coli* BL21 (DE3). To evaluate *in vivo* immune response of rMaHsp70, we administered intraperitoneal (IP) injection, and demonstrated that rMaHsp70 stimulated *M. amblycephala* immune activity by inducing the expression of *HSP70*, *HIF-1α*, *HSC70*, *CXCR4b*, *TNF-α* and *IL-1β* mRNAs in liver, headkidney, spleen and gill, as well as SOD, glutathione, lysozyme and interferon alpha proteins in serum and liver. The effect of rMaHsp70 as adjuvant against *Aeromonas hydrophila* was assessed by injecting a mixed vaccine of rMaHsp70 and *A. hydrophila* (*A. hydrophila*/Hsp70) into *M. amblycephala*, and the relative percent survival (RPS) in the *A. hydrophila*/Hsp70 group was 75% compared to 50% in the *A. hydrophila*/PBS group. Furthermore, rMaHsp70 also promoted the proliferation and suppressed apoptosis in *M. amblycephala* fin cells (MAF) in a dose-dependent manner. Taken together, these results suggest that rMaHsp70 can induce organic immune response and improve environmental tolerance.

© 2014 Elsevier GmbH. All rights reserved.

Introduction

Heat shock protein (Hsp) expression is regulated by environmental and physiological stress and non-stress factors (Tavaria et al. 1996; Yamashita et al. 2010). Hsps are classified into different families by molecular mass: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and the small Hsp families, and the amino acid sequences of each family are strongly conserved, especially the Hsp70 family (Muchowski and Wacker 2005; Shaner and Morano 2007).

Hsp70s play important roles in immune responses against tumor, bacterial and viral targets (Moroi et al. 2000; Li et al. 2006; Flechtner et al. 2006; Nishikawa et al. 2008). They can form complexes with various antigenic peptides through the polypeptide binding domain (Zhu et al. 1996). Srivastava has provided the evidence in mouse tumor models that vaccination with Hsp70 protein induces cancer-specific immunity and antigen-specific cytotoxic T lymphocyte activation by a mechanism involved antigen-presenting cells (APCs) (Srivastava 2002). Recent studies have indicated that the targeting of antigen to APCs through Hsp70s is a useful strategy to induce efficient cross-presentation, and it has been investigated for cancer immunotherapy (Takakura et al. 2007). Hsp70s can activate the innate immunity through CD40, Toll-like receptor (TLR)-2 and TLR-4 with cofactor CD14 and induce cytokine secretion from dendritic cells (Basu et al. 2000; Somersan et al. 2001; Srivastava 2002; Gross et al. 2003a,b).

In fish, several cDNA sequences encoding HSP70 from *Megalobrama amblycephala* (Ming et al. 2010), *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Oreochromis niloticus* (He et al. 2009), *Macrobrachium rosenbergii* (Liu et al. 2004), and *Danio rerio* (Lele et al. 1997) have been described. These studies focus on the expression of HSP70 under different environmental conditions, such as heat shock. Although Hsp70 plays a prominent role in stress response, there is little information about its immunological activity in fish.

* Corresponding author at: Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Fishery, Huazhong Agricultural University, 430070 Wuhan, PR China. Tel.: +86 027 87282113; fax: +86 027 87282114.
E-mail address: hbauwhl@hotmail.com (H.-L. Wang).

http://dx.doi.org/10.1016/j.imbio.2014.07.012
0171-2985/© 2014 Elsevier GmbH. All rights reserved.

M. amblycephala (Wuchang bream) as a freshwater fish has been cultured for half a century, however, intensive aquaculture has brought lots of problems, such as crowding, hypoxia, viral and bacterial invasion. In recent years, disease outbreaks have been reported to occur in *M. amblycephala* in China, resulting from the etiological *Aeromonas hydrophila* (Cipriano et al. 1984; Xia et al. 2012). In the present study, recombinant *M. amblycephala* Hsp70 protein (rMaHsp70) was purified from *E. coli* BL21 (DE3) and used to study the immune response *in vivo* and *in vitro*. In addition, rMaHsp70 can be used as adjuvant against *A. hydrophila*.

Materials and methods

Experimental fish

M. amblycephala (mean weight 25 g) were obtained from Ezhou breeding base in Hubei province, China. The fish were acclimated for two weeks and then used in the present study.

Cloning of 5′-flanking region of HSP70

Thermal asymmetric interlaced (TAIL)-PCR (Liu and Whittier 1995) was used to amplify 5′-flanking region of *HSP70*. In brief, total genomic DNA was extracted from *M. amblycephala* fin by traditional isopropanol precipitation method (Ciulla et al. 1988). Each special nest primers (SP1–SP3) and the corresponding arbitrary degenerate primers (AD1–AD3) were designed (Table 1). PCRs were performed with special reaction system and conditions (Supplement Tables 1 and 2). PCR products were cloned into pGEM-T Easy vector (Promega, Wisconsin, USA) and subsequently sequenced.

Promoter analysis

To predict putative *cis*-acting elements in the promoter, MatInspector version 9.0 was carried out with optimized matrix similarity (0.8, http://www.genomatix.com) (Cartharius et al. 2005).

Expression vector construction of the recombinant protein

Total RNA was isolated from *M. amblycephala* liver using TRIzol Reagent (Life Technologies, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized by M-MLV Reverse Transcriptase Kit (Promega, Wisconsin, USA) as follows: total volume of 15 μL reaction mixture, which contained 2 μg of total RNA and 1 μg of oligo $(dT)_{16}$ primer was incubated at 70 °C for 5 min to denature RNA secondary structure and then cooled on ice immediately. The following components in a total volume of 25 μL were added to the above reaction including 2 μL of 10 mM dNTPs, 5 μL of 5× M-MLV buffer, 200 units of M-MLV RT and 20 units of RNase inhibitor. The reverse transcription reaction was extended at 42 °C for 60 min. The open reading frame of *M. amblycephala HSP70* was amplified and directly cloned into pET-28a between *Nde* I/*Xho* I sites by E-HSP70-F/R primers (Table 1). The recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) to express recombinant *M. amblycephala* Hsp70 protein (rMaHsp70).

Expression and purification of recombinant protein

Recombinant *E. coli* BL21 (DE3) cells were cultured in LB medium at 15 °C and 37 °C, and induced with different IPTG concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM) for 6 h until OD_{600} reached 0.6–0.8. Cells were suspended in lysis buffer (20 mM Tris–HCl, 500 mM NaCl, 5 mM imidazole, pH 7.9) after centrifugation at 4000 × *g* for 5 min, and disrupted by sonication and centrifuged for 30 min (4 °C, 12,000 g). His-tagged rMaHsp70 was purified by affinity purification using 6× His-tagged Purification Kit (Cowin Biotech, Beijing, China) in accordance with the manufacturer's instructions.

Endotoxin (lipopolysaccharide, LPS) was removed from the purified rMaHsp70 by ToxinEraser Endotoxin Removal Kit (GenScript, Nanjing, China), and the remained endotoxin was measured using Endotoxin Assay Kit (GenScript, Nanjing, China) according to manufacturer's instructions. Then rMaHsp70 was confirmed by western blot using the rabbit Hsp70 polyclonal antibody (Boster, Wuhan, China).

ATPase assay

Hsp70s structurally possessed an N-terminal nucleotide-binding domain (NBD) or ATPase domain, with a weak ATPase activity which can be used to analyze activity of rMaHsp70 (Rothman 1989). The basal ATPase activity of rMaHsp70 was determined according to the method of Baykov with small changes (Baykov et al. 1988). Briefly, 2 μL of rMaHsp70 was mixed with 100 μL reaction mixture containing 30 mM TBST, 20 mM KCl, 1 mM $MgCl_2$, 2 mM DTT and 0.1 mM ATP. The mixture was then incubated at 40 °C for 10 min, followed by adding 10 μL 55% perchloric acid to stop the reaction. After centrifugation, the supernatant liquor of 80 μL was used to determine the inorganic phosphorus by Pi Measure Kit (Jiancheng, Nanjing, China). Absorbance at 618 nm was recorded and then converted to the concentration of released inorganic phosphate through a standard curve. As a control, rMaHsp70 inactivated by boiling at 100 °C for 30 min was also used in the assay.

Assay of immune activity in vivo

The purified rMaHsp70 was diluted in PBS to the final concentration of 0, 0.42 (R, endotoxin removed, and concentration was 368.45 EU/mg), 1.0 (R, 104.32 EU/mg) and 1.0 mg/mL (endotoxin not removed, 6080.23 EU/mg). Fish were randomized into four groups (five fish each group) and conducted IP injection with 100 μL rMaHsp70. The fish were fed normally in the next 24 h, and then liver, headkidney, spleen, gill and serum were sampled for analysis of expression of immune related genes including *HSP70*, *HIF-1α*, *HSC70*, *CXCR4b*, *TNF-α* and *IL-1β*, and enzyme activities or concentrations of SOD, malonaldehyde (MDA) and glutathione (GSH), lysozyme (LYZ) and interferon alpha (IFNα). The enzyme activities or concentrations of MDA, GSH and SOD were measured as described previously (Utley et al. 1967; Sedlak and Lindsay 1968; Elstner and Heupel 1976).

Vaccination

The *A. hydrophila* were isolated from diseased *M. amblycephala* in Dongxi Lake (Wuhan, China) and were cultured in LB medium to OD_{600} of 0.8, and suspended in PBS containing 0.5% formalin. The cells were incubated at 28 °C for 48 h, washed several times with PBS, and then adjusted cells to 1×10^9 CFU/mL through microscopic counting method.

To determine the adjuvant effect of rMaHsp70, two vaccine formulations were prepared: (1) 2 mL *A. hydrophila* vaccine (1×10^8 CFU/mL) alone; (2) 2 mL *A. hydrophila* vaccine (1×10^8 CFU/mL) mixed with rMaHsp70, which contained 105 μg/mL rMaHsp70 and 200 μL 10× Binding Buffer (100 mM KCl, 10 mM $MgCl_2$, 10 mM ADP) (Blachere et al. 1997). The mixture was incubated at 37 °C for 2 h. The two vaccines were pre-injected into four fish to ensure that the vaccines were sublethal. IP injection into *M. amblycephala* ($N = 15$) was performed using 100 μL *A. hydrophila*/rMaHsp70, *A. hydrophila*/PBS and PBS as described above, respectively. At 20 days post-vaccination, *M.*

Download English Version:

https://daneshyari.com/en/article/2182956

Download Persian Version:

https://daneshyari.com/article/2182956

Daneshyari.com