THE SMALL HEAT SHOCK PROTEINS STRUCTURE IN *CARASSIUS GIBELIO* AND *ONCORHYNCHUS MYKISS*

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Keywords: HSP27, *Carassius*, *Oncorhynchus*.

Abstracts: Heat shock proteins (HSP) are a family of proteins expressed in response to a wide range of biotic and abiotic stress factors. They are thus also referred to as stress proteins. Their extraordinarily high degree of identity at the amino acid sequence level and the fact that this cellular stress response has been described in nearly all organisms studied, make this group of proteins unique. The main aspects of this experiment were to identify the structural differences of small heat shock proteins between two different species of fishes *Carassius gibelio* Bloch. 1782 and *Oncorhynchus mykiss* Walbaum 1792.

INTRODUCTION

Heat shock proteins (HSPs) are a group of intracellular proteins that have an unusually high degree of identity at the amino acid level, among diverse organisms. As this family of proteins is induced by stressors other than heat, they are also commonly referred to as 'stress proteins' in the literature. The term stress proteins also may refer to several other groups of proteins that respond to stressors. For example, metallothioneins, which are expressed in response to heavy metal exposure, or cytochrome P450 enzymes, or HSPs, all may be considered as stress proteins. In contrast to the general nature of the term stress proteins, the HSP nomenclature is more commonly used in naming, and in reference to, specific HSPs. This review is concerned only with stress proteins that, to our knowledge, are HSPs. Thus, the HSP nomenclature is used. In the last three decades, there has been an exponential increase in the interest and research activity concerning the description, classification and functional significance of these proteins. Heat shock proteins are constitutively expressed in cells to maintain a number of critical cellular processes relating to protein folding, fidelity and translocation. These proteins also are induced in cells in response to a variety of stressors and enhance survival by protecting vital cellular functions (Iwama et al., 1998)

By using such purified heat shock mRNA fractions, HSP genes were among the first to be cloned in eukaryotes. Subsequently, nucleotide sequences became available and the unusually high degree of similarity in certain HSP groups, among diverse organisms, became known.

The naming of HSPs are generally based on their molecular mass (kilodaltons, kDa) as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Heat shock proteins are also grouped according to function (e.g. chaperonin), DNA sequence, and antibody cross-reactivity. The commonly used categories are: 100 kDa; 90 kDa; 70 kDa; 60 kDa; and the 16-30 kDa group, and are usually referred to as HSP100, HSP90, HSP70, HSP60, and the low molecular weight (LMW) class of proteins, respectively (Morimoto et al., 1994).

Heat shock protein studies involving the whole fish are few, and the majority of these have investigated the effects of heat shock. While most of these studies have subjected the organism to various treatments, some have experimentally treated tissues such as blood cells or fin cells extracted from the fish. Koban et al. (1991) described the induction of HSPs in the gill, liver, heart, erythrocytes, skeletal muscle and brain of *Fundulus heteroclitus*, *Cyprinodontidae* that were exposed to elevated temperatures. Dyer et al. (1991) studied HSP induction in the gill, striated muscle and brain of the fathead minnow subjected to heat shock. Dietz and Somero (1992) observed enhanced synthesis of HSP90 in the brain of two species of *Gillichthys mirabilis* and *G. seta*) held at elevated temperatures. Dietz and Somero (1993) also examined HSP90 synthesis patterns in response to heat shock of four marine species: *Enophrys bison*, *Cottidae*; *Leptocottus armatus, Cottidae*; *Citharichthys stigmaeus, Bothidae*; and *Parophrys vetulus, Pleuronectidae*. Mazur, in 1996 showed increased levels of HSP70 in gill, liver, anterior kidney, and muscle tissues of *Oncorhynchus clarki, Salmonidae* exposed to a 2 h heat shock of 10 °C (12.4-22.4 °C). HSP70 increased in all tissues after recovery for 1 h (except the muscle), and 48 h after being returned to the control temperature. In that study, a signifcant increase in HSP30 also was observed in gill tissue after recovery for 1 h. In a separate experiment, HSP70 in the red blood cells from cutthroat trout exposed to a 2 h heat shock of 16.2 °C (6.2-22.4 °C) were unchanged after recovery for 1 h, but were significantly elevated at 5 days after being returned to the control temperature (Mazur, 1996). This rather prolonged response also was seen in gill tissue of the same species exposed to a 2 h, 15 °C heat shock (7.4-22.4 °C), where increased HSP70 levels were observed up to 3 weeks after being returned to control temperatures (Mazur, 1996). In contrast, to acute studies of step-changes in temperature, *Carassius auratus, Cyprinida*e that were acclimated for at least 5 weeks to 10 °C and 30 °C showed higher concentrations of a new 65 kDa protein in the brain, liver and muscle tissues only in the warmer 30 °C water (Kikuchi et

al., 1993). That novel 65 kDa protein was distinct in characteristics from the HSP70 family of proteins, in that it had no affinity to ATP and did not react to antibodies raised against this novel protein.

The present paper has like principal aim, the structure of small heat shock protein 27 for individuals of *Carassius gibelio* and *Oncorhynchus mykiss*, two species with different abiotic conditions expressed in different temperature necessities and temperature adaptability.

MATERIALS AND METHODS

The experimental material was represented by 5 individuals from both species (*Carassius gibelio* Bloch, 1782 and *Oncorhynchus mykiss* Walbaum 1792). The sampling process followed to obtain approximately 3 cm long tissue samples from the dorsal muscle. The samples were kept in absolute ethanol at 4^{o}C.

DNA was automatically isolated and purified using the Maxwell 16 system (Promega) based on a standard elution volume (SEV). The purified total DNA's quantity and purity was electrophoretic and spectrophotometric tested.

The PCR was performed in total reaction volume of 25 µl using two complementary specific primers HSP27F, HSP27R (Wang et al., 2007) and GoTaq Green Master Mix (Promega). The gene's amplifying reaction took place at 58^{o}C as primer's aligning temperature, and a number of 30 replication cycles. To confirm the obtained sequence, PCR products were run on a 1,5% agarose gel containing ethidium bromide.

Towards sequencing, the codifying gene for HSP synthesis information, for both analyzed species, we used the same primers with 10µM concentration.

The PCR products were direct sequenced using an 8 capillaries Beckman Coulter 8000 sequencer.

Binding the direct and reverse chains for each individual was performed using SeqBuilder (Lasergene 8) software.

The analysis of small heat shock proteins from two species of fishes prussian carp (*Carassius gibelio* Bloch. 1782) and rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) has been carried out, using EditSeq for trasnlation and Protean Lasergene v8 for protein structure identification.

RESULTS AND DISCUSSIONS

The resulted sequences were forward translated into amino acids chains based on a vertebrate nuclear genetic code, using the EditSeq module (Lasergene 8) as show in Figure 1.

Figure 1 Nucleotide and deduced amino acid sequence for *Carassius'* Hsp27 (points represents the terminal sites)

The deduction of the amino acids strain reveals the existence of 343 units (Table 1)

Table 1 The amino acids number, percentage and frequency for *Carassius'* Hsp 27

Amino Acid(s)	Number count	% by weight	% by frequency	Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	114	38.81	33.24	M Met	9	2.98	2.62
Acidic (DE)	19	5.91	5.54	N Asn	11	3.17	3.21
Basic (KR)	51	18.69	14.87	P Pro	26	6.37	7.58
Polar (NCQSTY)	98	26.34	28.57	Q Gln	9	2.91	2.62
Hydrophobic (AILFWV)	111	31.85	32.36	R Arg	31	12.22	9.04
A Ala	22	3.95	6.41	S Ser	28	6.15	8.16
C Cys	18	4.69	5.25	T Thr	24	6.12	7.00
D Asp	8	2.32	2.33	V Val	11	2.75	3.21
E Glu	11	3.58	3.21	W Trp	10	4.70	2.92
F Phe	12	4.46	3.50	Y Tyr	8	3.29	2.33
G Gly	11	1.58	3.21	B Asx	0	0.00	0.00
H His	18	6.23	5.25	Z Glx	0	0.00	0.00
I Ile	14	4.00	4.08	X Xxx	0	0.00	0.00
K Lys	20	6.47	5.83	. Ter	13	0.00	3.79
L Leu	42	12.00	12.24				

Subsequently, the protein structure was analyzed, (Figure 2) and alpha regions, beta regions and turn regions have been determined using the Protean (Lasergene 8) software and two methods Garnier-Robson (Garnier et al., 1978) and Chou-Fasman (Chou and Fasman, 1978); hydrophilicity plot graphic representation (Kyte and Doolittle, 1982), flexible regions (Karplus şi Schultz, 1985), antigenic index (Jameson and Wolf, 1988) and surface probability plot (Emini et. al., 1985).

The polypeptidic chain structure analysis for *Carassius gibelio* (Figure 1) shows that the there are no major differences between Garnier-Robson (GR) and Chou-Fasman (CF) methods. Regarding the alpha helical regions; thereby, for the GR method we determined the existence of 13 alpha helical regions and 9 for the CF method, with a surface partial superposition comparative between methods. Regarding the beta folded regions, for both methods, are detected the 25 beta folded region (GR) and only 7 (CF), at the starting point of the polypeptidic chain, with the distinction given by those regions' surface size differences, determined based on the two methods.

Regarding the turn regions, for the first method (GR), 29 such zones are given, while by CF method 25 regions can be detected; the difference is that in the second method, they are presented as regions with larger surfaces.

Based on the hydrophobicity graphic, build using the Kyte-Doolittle model (Kyte and Doolittle, 1982), 14 hydrophobic and 9 hydrophilic zones were determined.

From the antigenic index graphic, calculated based on the Jameson–Wolf model (Jameson and Wolf, 1988), 21 regions with antigenic potential were observed.

Analyzing the polypeptidic chain structure for *Carassius gibelio* (Figure 2) shows that there are major differences between Garnier-Robson (GR) and Chou-Fasman (CF) methods. Regarding the alpha helical regions; thereby, for the GR method we determined the existence of 13 alpha helical regions and only 5 for the CF method, with a surface partial superposition comparative between methods., For both methods recording the number of beta folded regions, are detected 4 regions (GR) and 10 using the CF model, at the starting point of the polypeptidic chain.

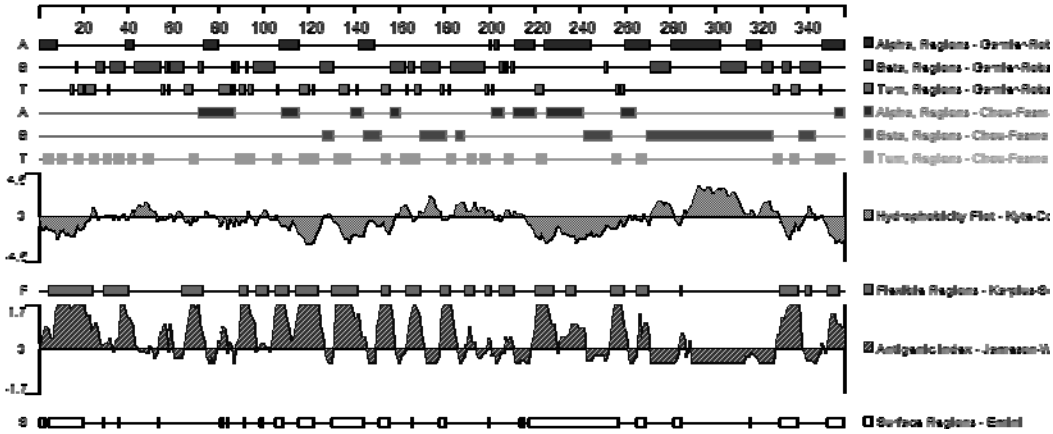

Figure 2 The analysis of Hsp 27 protein structure for *Carassius gibelio*

Also, the isoelectric point was recorded at 9.99 (Figure 3), using the Protean module of DNAstar Lasergene V8 software.

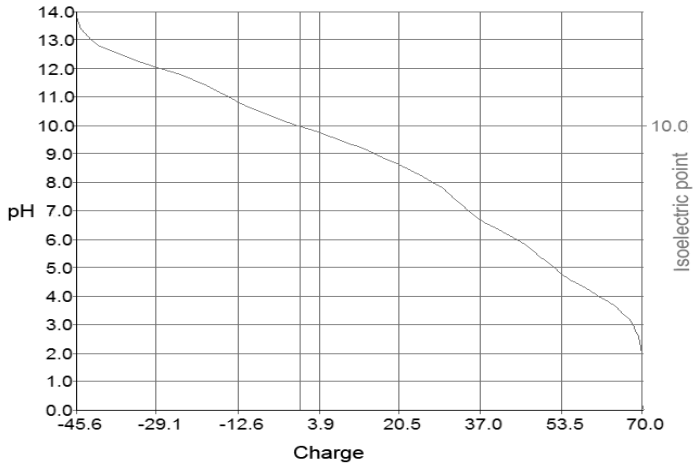

Figure 3 The titration curve for *Carassius* Hsp 27 protein fragments

Analyzing in Protean (Lasergene v8) software the SDS-PAGE of *Carassius gibelio* Hsp 27 protein (Figure 4), cut with three different protease, Trypsin (TRYT), which produce 46 fragments, first with molecular weight of 146,17D and isoelectric point (I.p.) at 9 and the last one 3383.25 m.w. (5.51 I.p), hydroxylamine (HYDR) with 6 cuts between 920.12 m.w. (12.40 I.p.) and 14932.45 m.w. (11.54 I.p.); Cyanogen bromide (CNBr), Cys methylated with 24 cuts between 160.16 m.w. (5.55 I.p.) and 4124,69 (8.99 I.p.)

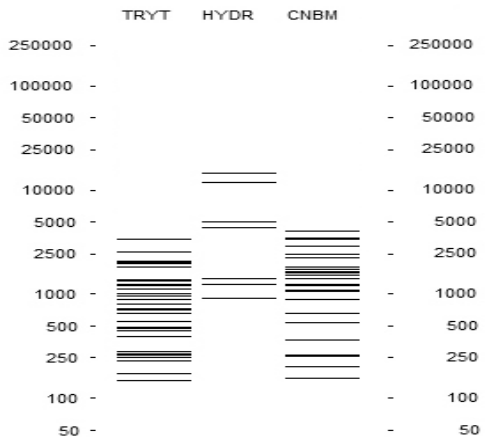

Figure 4 Hsp 27 protein fragments electrophoresis of for *Carassius gibelio*
(TRYT= Trypsin, HYDR= hydroxylamine, CNBM=Cyanogen bromide Cys methylated)

The sequencing process for of *Oncorhynchus* Hsp 27 gene sequence (Figure 5), the deduction of amino acids chain, helps to identify 350 units (Table 2) using the SeqBuilder (Lasergene v.8) module.

Regarding the turn regions (Figure 6), for the first method (GR), 17 such zones are given, while by CF method 14 regions can be detected; the difference is that in the second method, they are presented as regions with larger surfaces.

Based on the hydrophobicity graphic, build using the Kyte-Doolittle model (Kyte and Doolittle, 1982), 14 hydrophobic and 9 hydrophilic zones were determined.

From the antigenic index graphic, calculated based on the Jameson–Wolf model (Jameson and Wolf, 1988), 21 regions with antigenic potential were observed.

The isoelectric point has a value of 9.1 corresponding to pH7 (Figure 7)

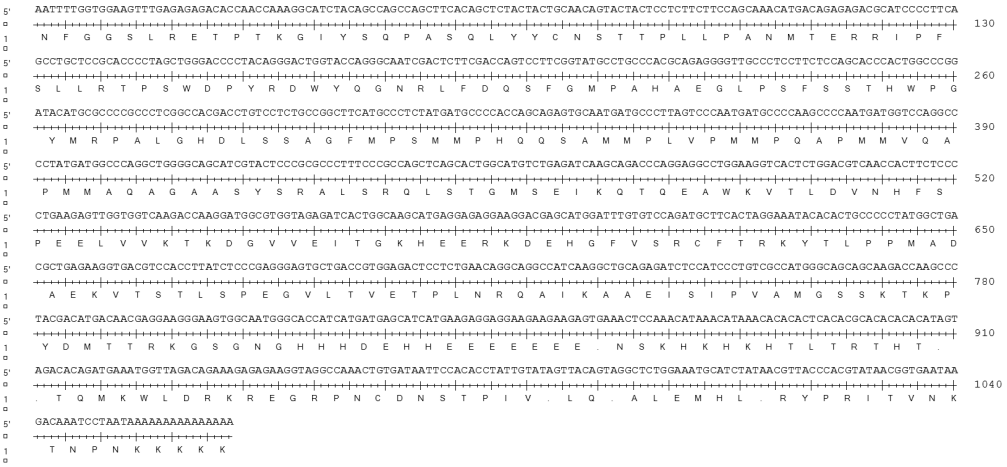

Figure 5 Nucleotide and deduced amino acid sequence for *Oncorhynchus’* Hsp27
(points represents the terminal sites)

Table 2 The amino acids number, percentage and frequency for *Oncorhynchus'* Hsp 27

Amino Acid(s)	Number count	% by weight	% by frequency	Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	111	38.10	31.71	M Met	20	6.63	5.71
Acidic (DE)	37	11.64	10.57	N Asn	13	3.75	3.71
Basic (KR)	44	15.66	12.57	P Pro	29	7.11	8.29
Polar (NCQSTY)	99	27.29	28.29	Q Gln	15	4.86	4.29
Hydrophobic (AILFWV)	84	22.84	24.00	R Arg	20	7.89	5.71
A Ala	22	3.95	6.29	S Ser	29	6.38	8.29
C Cys	3	0.78	0.86	T Thr	29	7.41	8.29
D Asp	12	3.49	3.43	V Val	15	3.76	4.29
E Glu	25	8.15	7.14	W Trp	5	2.35	1.43
F Phe	9	3.35	2.57	Y Tyr	10	4.12	2.86
G Gly	20	2.88	5.71	B Asx	0	0.00	0.00
H His	17	5.89	4.86	Z Glx	0	0.00	0.00
I Ile	9	2.57	2.57	X Xxx	0	0.00	0.00
K Lys	24	7.77	6.86	. Ter	6	0.00	1.71
L Leu	24	6.86	6.86				

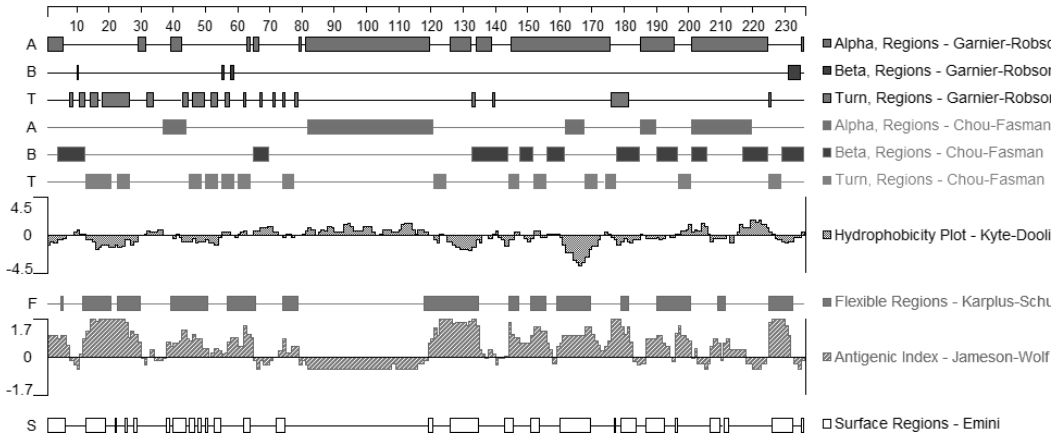

Figure 6 The analysis of Hsp 27 protein structure for *Oncorhynchus mykiss*

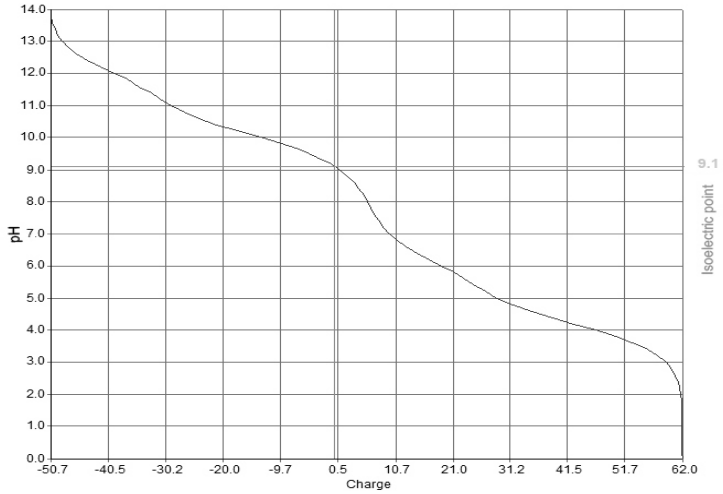

Figure 7 The titration curve for *Oncorhynchus* Hsp 27 protein

Analyzing the SDS-PAGE of *Oncorhynchus mykiss* Hsp 27 protein (Figure 8) in Protean module, cut with three different protease, results that Trypsin (TRYT), produce 21 fragments, first with molecular weight of 146,17D and isoelectric point (I.p.) at 9 and the last one 9697.46 m.w. (5.41 I.p), hydroxylamine (HYDR) with 1 cut of 25767.84 m.w. (6.87 I.p.); Cyanogen bromide (CNBr), Cys methylated with 17 cuts between 149.20 m.w. (5.55 I.p.) and 5935.58 (5.61 I.p.).

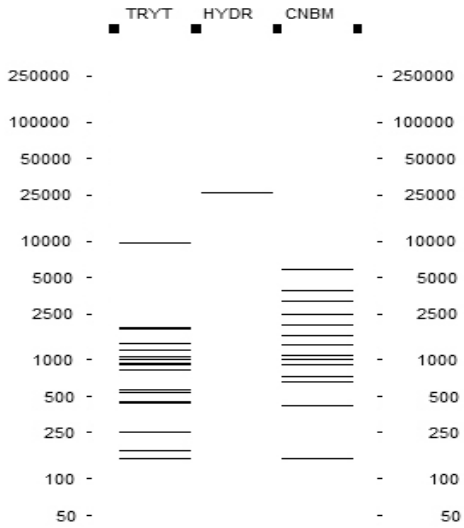

Figure 8 Hsp 27 protein fragments electrophoresis of for *Oncorhynchus*
(TRYT= Trypsin, HYDR= hydroxylamine, CNBM=Cyanogen bromide Cys methylated)

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