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Preferential activation of HSF-binding activity and *hsp70* gene expression in *Xenopus* heart after mild hyperthermia

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Abstract We have examined the effect of mild hyperthermia on the pattern of heat shock transcription factor (HSF) binding activity, heat shock protein 70 (*hsp70*) and *hsp30* gene expression and protein denaturation in selected tissues of adult *Xenopus* namely, heart, hind limb muscle, eye, liver and spleen. In these studies it was found that heart tissue was the most thermally sensitive of all of the tissues examined since maintenance of adult frogs at 26°C resulted in a preferential activation of HSF binding. Thus, heart has a lowered set point temperature for HSF activation compared to the other tissues examined. At 30°C HSF activation was observed in all of the tissues examined. Heart HSF activation at 26°C was correlated with an increase in *hsp70* mRNA and Hsp70 protein accumulation. At 28°C the largest amount of *hsp70* and *hsp30* mRNA accumulation was detected in heart and skeletal muscle compared to other tissues while *hsp70* mRNA accumulation was relatively low in spleen and *hsp30* mRNA accumulation was not detectable in eyes, liver and spleen. Incubation of adult frogs at 30°C resulted in enhanced *hsp70* and *hsp30* mRNA accumulation in all of the tissues. Finally, we have used differential scanning calorimetry (DSC) to compare the temperatures at which protein denaturation occurs in heart and liver tissue. The onset of protein denaturation (T_0) occurred approximately 8.5°C lower in heart compared to liver. Also the midpoint of the DSC profile ($T_{1/2}$) was approximately 10.4°C lower in heart than in liver. Thus, heart proteins are generally more thermolabile than proteins in liver tissue. Taken together these data suggest that heart is more sensitive than the other tissues examined with respect to moderate increases in environmental temperature.

INTRODUCTION

Heat shock-induced activation of the expression of a set of heat shock protein (*hsp*) genes giving rise to the accumulation of *hsp* mRNA and the synthesis of Hsps is characteristic of essentially all organisms examined (reviewed by Atkinson and Walden 1985; Parsell and Lindquist 1993; Morimoto et al 1994). The expression of *hsp* genes has been shown to be regulated at the transcriptional, post-transcriptional and translational level. These stress proteins appear to act as molecular chaperones and are involved in protein folding and transport across the intracellular membranes (Hightower et al 1994; Morimoto et al 1994). A number of studies have shown that the presence of unfolded protein during stresses such as heat shock may result in the transcription of *hsp* genes via activation of heat shock factor (HSF) (reviewed in Morimoto et al 1994). In one model the association of monomeric HSF with Hsp70 in the cytoplasm is disrupted by heat shock

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due to the increase in the concentration of unfolded protein. While Hsp70 binds to unfolded protein, HSF trimerizes and then interacts with the heat shock element (HSE) found in the promoter regions of *hsp* genes. This is followed by a series of events leading to the transcription of *hsp* genes such as *hsp70*. Most of the work on the heat shock response has been carried out in cultured cell systems or organ cultures. While there are a substantial number of studies examining the effect of hyperthermia on *hsp* gene induction in vivo in mammals (reviewed in Brown 1990; Kochevar et al 1991; Knowlton 1994, 1995; Yellon and Marber 1994; Mestril and Dillman 1995) only a few studies have examined this phenomenon in poikilothermic vertebrates such as salamander and fish (Easton et al 1987; Rutledge et al 1987; Bols et al 1992; Dietz and Somero 1993; Dietz 1994; White et al 1994). These types of studies are important given that the body temperature of poikilotherms varies with environmental temperature in contrast to homeothermic organisms which have mechanisms in place to try to maintain a constant temperature.

Xenopus laevis has been used as a model system to examine *hsp* gene expression. However, most of this work has been carried out in oocytes, early embryos and in tissue culture cells (reviewed in Heikkila et al 1997; Gordon et al 1997; Mercier et al 1997). To date, there has been only a single report examining the effect of in vivo hyperthermia on *hsp* gene expression in adult tissues of *Xenopus laevis* (Bienz 1984a). In this latter report adult frogs were exposed to relatively high incubation temperatures (33°C for 3 h). Here we have examined the effect of different incubation temperatures ranging from 22°C to 30°C on the activation of HSF binding activity, *hsp70* and *hsp30* gene expression and protein denaturation in selected tissues in adult *Xenopus laevis*.

MATERIALS AND METHODS

Heat shock experiments

Mild whole body hyperthermia was performed by placing adult *Xenopus laevis* (2–3 years old) in waterbaths pre-equilibrated at the desired temperatures (22°C – 30°C) for periods of time ranging from 25 min to 5 h. The animals were then killed and different tissues including heart, muscle, liver, eyes and spleen were isolated immediately, frozen and then stored at –80°C.

DNA mobility shift assay

DNA mobility shift assays were performed essentially as described by Ovsenek and Heikkila (1990). For protein extracts, neurula stage embryos or adult tissues were homogenized in Buffer C (50 mM Tris-Cl (pH 7.9), 20% glycerol, 50 mM Kcl, 0.1 mM EDTA, 2 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin (Dignam et al 1983). After centrifugation at 15 000 g for 5 min at 4°C, the supernatants were frozen in liquid nitrogen. DNA-binding reactions with either embryonic or adult tissue samples contained 10 μl protein extract. For these experiments, the protein equivalency was determined by the Biorad protein assay followed by visualization of the proteins on Coomassie staining of SDS-polyacrylamide gels. Extract volumes were adjusted so that equal protein amounts were added to each binding reaction. HSE oligonucleotide probes used in our assays were as described in Ovsenek et al (1990). Binding reactions were performed in the presence of 1 μg poly (dI-dC), 10 mM Tris (pH 7.8), 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 5% glycerol, in a final volume of 20 μl. Reactions were incubated on ice for 20 min and immediately loaded onto 5% non-denaturing polyacrylamide gels containing 6.7 mM Tris-Cl (pH 7.5), 1 mM EDTA, 3.3 mM sodium acetate. Gels were electrophoresed for 2.5 h at 150 V, dried and exposed overnight to X-ray film (Kodak X-omat 5).

RNA isolation

Total RNA was isolated from adult tissues by the GIT/CsCl centrifugation method as described by Chirgwin et al (1979) with some modifications (Ohan and Heikkila 1995). Briefly, the tissues were homogenized in 10 ml of 4 M guanidine isothiocyanate and layered on top of 3.3 ml of 5.7 M cesium chloride solution and then centrifuged for 23 h at 30K rpm in an SW-41 Ti rotor (Beckman). The RNA pellets were recovered and purified by two consecutive ethanol precipitations.

Northern blot analysis

Fifteen μg of total RNA was electrophoresed on 1.2% formaldehyde agarose gels and transferred to nylon membrane (ICN, Mississauga, Ontario, Canada; Sambrook et al 1989). To ensure equal sample loading, RNA was visualized on formaldehyde agarose gels after ethidium bromide staining. The blots containing RNA were UV cross-linked with a GS-Gene linker (BioRad). The hybridization reactions were performed as described previously (Krone and Heikkila 1988) using ^{32}P-labeled *hsp70B* (Bienz 1984b) and *hsp30C* (Krone et al 1992) probes. The post-hybridization washes were performed under high stringency conditions followed by exposure to XAR-5 film at –70°C. The experiments were repeated three times and in some cases the probe was stripped from the blot and rehybridized with a different probe. Autoradiograms were scanned using an Apple Macintosh OneScanner and the data was analyzed using NIH Image Version 1.55 software.

Immunoblot analysis

Proteins were extracted as described by Smith (1986) employing the modifications of Winning et al (1989) and resolved by 2-D PAGE as previously described (Tam and Heikkila 1995). Molecular masses were determined by co-electrophoresis of molecular weight standards (BioRad). Proteins separated by 2-D PAGE which were destined for immunoblot analysis were electroblotted to nitrocellulose membrane (BioRad) at 120 V in 25 mM Tris, 192 mM glycine and 20% (v/v) methanol for 1.5 h. Blots were incubated overnight with 5% skim milk powder in tris buffered saline (50 mM Tris pH 7.5, 150 mM NaCl) with 0.05% Tween 20 (BioRad). Blots were incubated with a mouse monoclonal Hsp70 primary antibody (clone 3a3, Affinity Bioreagents) for 3 h and a horseradish peroxidase conjugated anti-mouse IgG (Boehringer Mannheim) secondary antibody for 1 h. Immunodetection using the Boehringer Mannheim Chemiluminesence Kit was carried out according to the manufacturer's instructions.

Differential scanning calorimetry

Liver and heart tissue were isolated and then frozen at −80°C. In preparation for differential scanning calorimetry, the tissues were homogenized in ice cold Hepes buffer (pH 7.4) saline solution (25 mM Hepes, 5.4 mM KCl, 137 mM NaCl, and 5 mM $MgCl_2$) with 0.5 mM PMSF. The samples were then scanned with a Microcal MC-2 differential scanning calorimeter (DSC), with 1.21 ml sample cells, heated from 10°C to 110°C at a scan rate of 1°C/min and at a tissue concentration of 80–100 mg/ml (wet tissue/buffer). Rescans were always obtained to check for reversibility and baseline curvature. The baselines were corrected for curvature and the shift in specific heat (△Cp) upon denaturation as previously described (Lepock et al 1993). The scans were smoothed using a polynomial least squares method and corrected for protein concentration. The data are plotted as excess heat capacity at constant pressure (Cp) vs. temperature.

RESULTS

In the present study we have examined the activity of the *Xenopus* HSF, employing DNA mobility shift analysis, in extracts from selected tissues of adult frogs subjected to mild hyperthermia. The synthetic oligonucleotide used in this study corresponds to the proximal HSE of the *Xenopus hsp70B* gene and has been used previously to study HSF activity in *Xenopus* embryos (Ovsenek and Heikkila 1990; Karn et al 1992). As shown in Figure 1, we did not detect any HSE-binding activity in selected tissues of adult frogs maintained at their normal temperature of 22°C. However, placement of adult frogs at 26°C for 25 min resulted in HSE-binding activity in heart

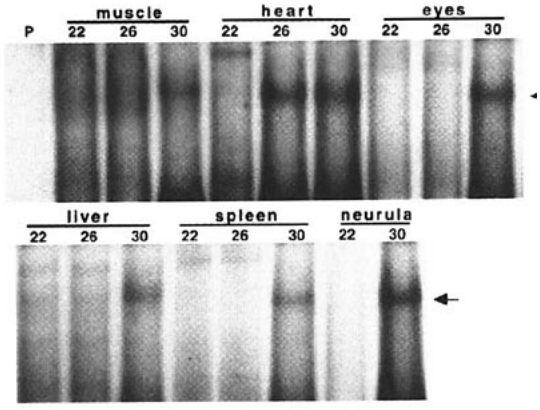

Fig. 1 Detection of heat inducible HSE–HSF binding activity in adult *Xenopus* tissues and neurula stage embryos. Adult *Xenopus* were maintained at either the control temperature (22°C) or heat shocked at either 26°C or 30°C for 25 min after which tissues were isolated. Neurulae were maintained at either the control temperature (22°C) or heat shocked at 30°C for 25 min. DNA mobility shift assays were performed using labelled double-stranded HSE oligonucleotide (P) mixed with whole cell extracts from either *Xenopus* tissues or neurulae. The arrow indicates the position of the HSE–HSF complex.

tissue, but not in eyes, liver, or spleen tissue. Occasionally hind limb muscle tissue displayed a very small amount of HSF binding. At 30°C all of the adult tissues examined displayed HSE-binding activity. The position of the HSE-binding activity in these autoradiograms was similar to that found with heat shocked neurula (Fig. 1) which has been characterized in previous studies (Ohan and Heikkila 1990; Karn et al 1992). The specificity of the HSE-binding activity in heart tissue from the 26°C-treated animals was assessed by competition experiments (Fig. 2). The addition of a 100-fold excess of unlabeled HSE oligonucleotide in the binding reaction resulted in a reduction of the retarded complex (compare lanes 2 and 3) whereas the presence of a 100-fold excess of unlabeled non-complementary AP1 oligonucleotide did not affect HSF–HSE interaction (compare lanes 2 and 4). These results suggest that placement of adult frogs at 26°C produces a preferential activation of HSF in *Xenopus* heart.

Given these results, we examined the effect of mild hyperthermia on the accumulation of *hsp70* mRNA in different tissues of adult *Xenopus* by Northern blot analysis employing the *Xenopus hsp70B* genomic clone. *hsp70* mRNA accumulation in the various tissues in response to whole body hyperthermia is shown in Figures 3 and 4. While maintenance of frogs at 22°C did not result in detectable *hsp70* mRNA accumulation, placement of frogs at 26°C for 5 h resulted in enhanced accumulation of *hsp70* mRNA in heart but not in hind limb muscle, eyes, liver or spleen. In these experiments, 5 h of 26°C treatment was required for maximum accumulation of

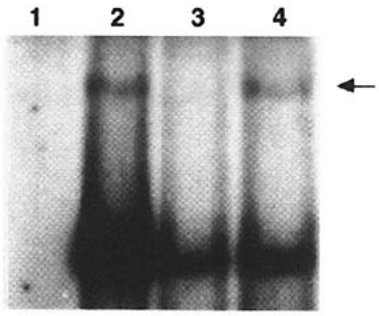

Fig. 2 Specificity of HSF–HSE binding activity in whole cell extract of adult *Xenopus* heart. A DNA mobility shift assay was performed using 1 ng of end-labeled HSE oligonucleotide (lane 1) and heart tissue extract (lane 2). Competition experiments were performed with a 100-fold excess of unlabelled HSE oligonucleotide (lane 3) or 100-fold excess of unlabelled non-complementary AP1 oligonucleotide (lane 4). Specific complexes are indicated with an arrow.

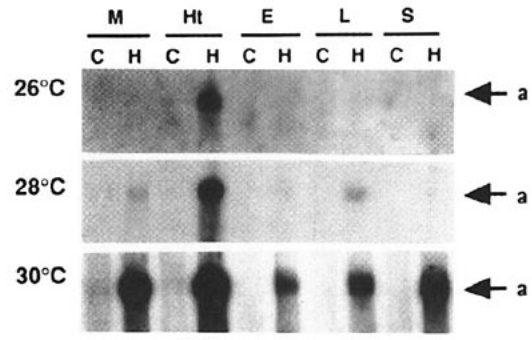

Fig. 3 Hyperthermia-induced accumulation of *hsp70* mRNA in adult *Xenopus* tissues. Whole body hyperthermia was provided at the indicated temperatures for 5 h. Total RNA isolated from the tissues was subjected to Northern blot analysis using the *Xenopus hsp70B* probe. E, eyes; L, liver; M, muscle; S, spleen; H, heart. *Hsp70* transcript size a = 2.4 kb.

hsp70 mRNA in heart tissue (data not shown). Exposure of frogs to 28°C resulted in the highest relative levels of *hsp70* mRNA in heart. Densitometric analysis revealed that hind limb muscle tissue contained approximately 55% of the levels of *hsp70* mRNA found in heart while eyes and liver had 35–40% and spleen had 10% (Fig. 4). At 30°C a stronger response with respect to *hsp70* gene expression was detectable in all of the tissues. The highest relative levels of *hsp70* mRNA occurred in heart and hind limb muscle with lower levels in eyes, liver and spleen. In separate experiments, we found that these results were not affected by the sex of the adult frogs (data not shown).

We also examined the effect of mild hyperthermia on the relative level of *hsp30* mRNA. Analysis of the effect of mild hyperthermia on *hsp30* gene expression revealed that *hsp30* mRNA accumulation was not detectable at temperatures ranging from 22°C to 26°C (Figs 5 and 6). However, a low level of *hsp30* mRNA accumulation was detectable at 28°C in muscle and heart but not in any of the other tissues examined. At 30°C the largest increase in *hsp30* mRNA accumulation occurred in muscle. Densitometric measurements determined that the relative level of hsp30 mRNA compared to muscle was 85% in heart, 65% in eyes, 55% in liver and 45% in spleen (Fig. 6).

This study has demonstrated that placement of adult *Xenopus* at 26°C results in the activation of HSF and the accumulation of *hsp70* mRNA in heart tissue but not in the other tissues examined. In order to determine whether there was an increase in the relative level of Hsp70/Hsc70 protein in heart tissue under these conditions we carried out Western blotting employing a human Hsp70 monoclonal antibody. This Hsp70 antibody which recognizes an epitope located within amino acids 504–617 of human Hsp70 reacts with both *Xenopus* Hsp70 and Hsc70. Comparison of the amino acid sequences of *Xenopus* Hsp70 (Bienz 1984b) and Hsc70 (Ali et al 1996) revealed a high homology with human Hsp70 in this region. To date we have not found an antibody that discriminates between the two *Xenopus* Hsp70 family members. As shown in Figure 7, heart tissue contains a greater amount of constitutive Hsp70/Hsc70 than found in liver. Furthermore, exposure of adult frogs to a temperature of 26°C for 5 h resulted in an increase in the relative level of Hsp70/Hsc70 in heart tissue but not in liver. Similar results were observed with other non-cardiac tissues (Tam and J. J. Heikkila, unpublished results).

The results of the above experiments suggest that heart tissue may be more sensitive to hyperthermia than the other tissues examined. Also, we have used DSC to determine the temperatures at which protein denaturation occurs in heart and liver tissue. As a protein or a domain within a protein undergoes thermal denaturation, there is an increase in heat absorption which is detected as an apparent excess specific heat (Cp). The profiles of excess Cp for heart and liver homogenate are given in Figure 8. The units for Cp are expressed per gram of tissue. Each complex profile consists of a number of overlapping endothermic peaks, indicating the contribution of a number of endothermic processes or transitions. The profile represents the sum of all cellular transitions including protein denaturation, DNA and RNA unfolding, and molecular aggregation and disaggregation. On the basis of relative specific heats and fractional composition, protein denaturation should predominate over other endothermic processes (Lepock et al 1993).

The profile for heart consists of a small peak, which appears as a shoulder, with a T_m approximately 35°C, the major peaks at T_m = 46°C, and several smaller peaks at higher temperatures. All transitions for both heart and

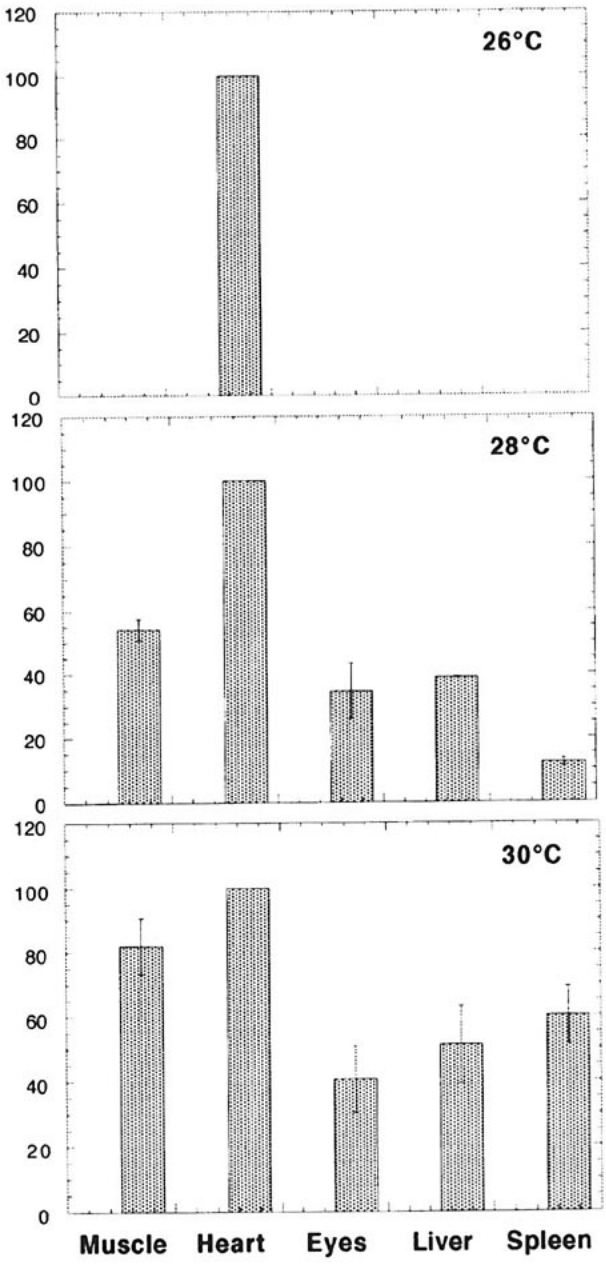

Fig. 4 Quantitation of heat-induced accumulation of *hsp70* mRNA in adult *Xenopus* tissues. Autoradiograms from several different experiments were scanned and the background values were subtracted from the values obtained for each lane. The values were then expressed as a percentage of the maximum mRNA accumulation (Y axis) which in this Figure was heart tissue. For each of the samples, the mean and standard error of the mean are indicated. The shaded boxes represent the heat shock samples at the temperature indicated at the top of each graph.

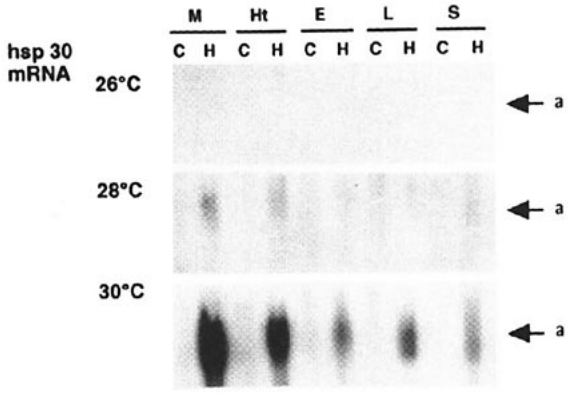

Fig. 5 Hyperthermia-induced accumulation of *hsp30* mRNA in adult *Xenopus* tissues. Total RNA isolated from the tissues was subjected to Northern blot analysis using the *Xenopus hsp30C* genomic clone. *hsp30* transcript size a = 1.1 kb.

liver are irreversible after scanning to 100°C. The profile for liver consists of the major peak at T_m = 70°C, two weaker transitions at lower temperatures which appear as shoulders on the main peak, and a high temperature transition at T_m = 85°C. In addition, the profile for liver has a broad exotherm, probably due to metabolism (Lepock et al 1993) centered near 38°C. A very weak metabolic exotherm may also occur in the profile for heart.

Two important parameters can be used to characterize these profiles: the onset of detectable denaturation (T_0), defined as the minimum temperature of first deviation from the baseline indicating an endothermic transition, and $T_{1/2}$, defined as the midpoint (temperature of half area) of the profile. The average value of T_0 from five heart scans is 29.3 ± 2.3°C (standard error). The corresponding value for liver is 37.8 ± 2.9°C. The value of T_0 for liver is more uncertain because of the presence of the metabolic exotherm. These values are several degrees higher than the minimum temperature at which protein denaturation could occur during long exposure to a constant temperature since denaturation is irreversible and dependent on both temperature and time of exposure. The DSC profiles are obtained at a scan rate of 1°C/min. Thus, only 3 min elapsed between 26°C, the minimum temperature for activation of HSF over a period of 25 min, and 29°C, the first detectable denaturation by DSC. The consequence of this is that the onset of denaturation is shifted upward by several degrees because of the relatively rapid scan rate employed (Ritchie et al 1994). The higher value of T_0 in liver indicates that the most thermolabile proteins in liver are more stable than the most thermolabile proteins in heart. The values of $T_{1/2}$, 58.8 ± 3.5°C for heart and 69.2 ± 2.3°C for liver, indicate that the average protein stability in liver is greater than that in heart.

DISCUSSION

We investigated the effect of mild hyperthermia, by raising the ambient water temperature from 22°C to 30°C, on HSF binding activity, *hsp70* and *hsp30* gene expression and protein denaturation in selected tissues of adult

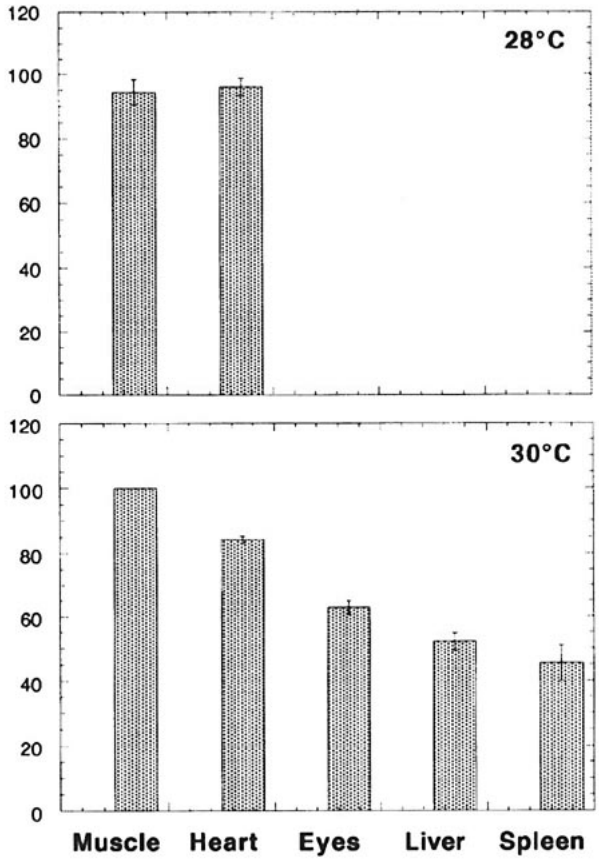

Fig. 6 Relative quantitation of heat-induced accumulation of *hsp30* mRNA in adult *Xenopus* tissues. Autoradiograms from several different experiments were scanned and the background values were subtracted from the values obtained for each lane. The values were then expressed as a percentage of maximum mRNA accumulation (Y axis) which was muscle tissue in the 30°C panel. It should be noted that for 28°C, in some experiments muscle contained the highest amount of *hsp30* mRNA while in other experiments heart had the maximum amount. The histogram reflects this situation. For each of the samples, the mean and standard error of the mean are indicated. The shaded boxes represent the heat shock samples at the temperature indicated at the top of each graph.

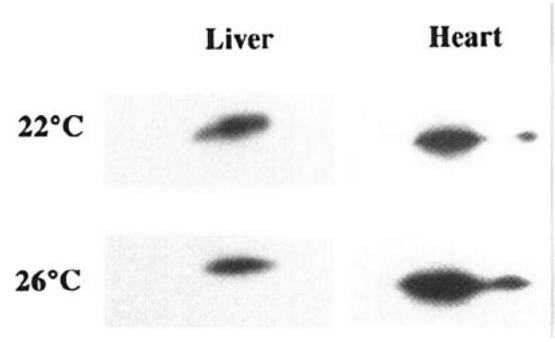

Fig. 7 Effect of mild hyperthermia on the relative levels of Hsp70/Hsc70 protein in adult heart and liver. Adult *Xenopus* were maintained at either 22°C or 26°C for 5 h. Total protein was isolated from the different tissues and 30 μg was subjected to 2-D SDS-PAGE followed by Western blotting as outlined in Materials and Methods. The blots were reacted against an Hsp70 antibody which recognizes both Hsp70 and Hsc70 family members.

Xenopus laevis. Interestingly, we found that heart tissue appears to be the most thermally sensitive tissue compared to hind limb muscle, liver, spleen and eyes. For example, placement of the adult frog at 26°C, which is within the normal temperature range of this animal (Wu and Gerhart 1991), resulted in preferential activation of HSF–HSE complex formation in heart tissue. The HSF–HSE complexes in heart were similar in migration compared to the previously characterized neurula embryos and oocytes (Ovsenek and Heikkila 1990; Karn et al 1992; Gordon et al 1997). Also competition experiments with excess unlabeled HSE oligonucleotide demonstrated that DNA sequence-specific binding activity occurred in heart tissue at 26°C. The detection of inducible HSF activity in heart tissue after a temperature shift from 22°C to 26°C is similar to earlier results with neurula embryos in which HSE-binding activity was first detectable at 27°C (Karn et al 1992). At 30°C HSE-binding activity was detectable in all of the tissues examined. These results suggest that the temperature set point for HSF activation in *Xenopus* heart tissue is lower than that found in the other tissues examined. Different HSF activation temperature set points have been reported in mouse tissue. For example, pachytene spermatocytes displayed a 7°C lower HSF1 activation temperature than somatic testis cell types and other tissues such as liver (Sarge 1995; Sarge et al 1995). Our results support the contention of Sarge (1995) that the temperature of HSF activation may not necessarily have a fixed value in a given species and that it can vary in a tissue-dependent manner. However, the mechanism(s) involved in determining the set point temperature of HSF activation in different cell or tissue types remains to be elucidated. One contributing factor which may be involved in the preferential activation of HSF in *Xenopus* heart as a result of mild hyperthermia is the increased heart rate compared to other tissues and the accompanying energy demand. While this aspect was not examined in the present study, the combined stress of increased contractions in heart tissue as well as the increase in body temeprature may explain why skeletal muscle tissue was not affected.

Induction of HSF binding activity in heart tissue at 26°C correlated with a preferential accumulation of *hsp70* mRNA. Enhanced accumulation of *hsp30* mRNA was not detectable at this temperature. Furthermore, at 30°C all tissues examined displayed increased relative levels of *hsp70* and *hsp30* mRNA compared to 22°C which correlated with HSF-binding activity. The induction of HSF binding activity and *hsp70* mRNA accumulation in heart tissue at 26°C was also associated with the enhanced accumulation of Hsp70/Hsc70 protein. It is

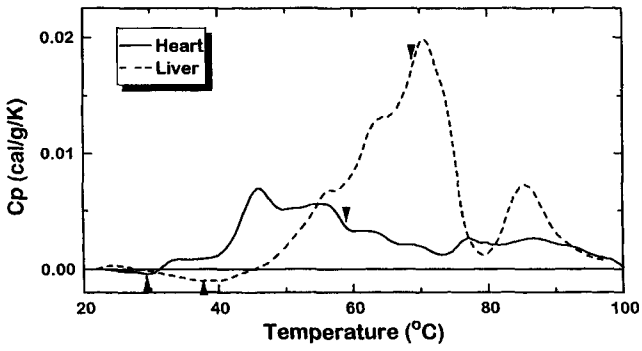

Fig. 8 Differential scanning calorimeter (DSC) analysis of *Xenopus* heart and liver tissue protein homogenates. Total protein was isolated from frozen tissues previously excised from *Xenopus* maintained at 22°C as described in Materials and Methods. Data are plotted as heat capacity of proteins at constant pressure (Cp) vs temperature. The arrows pointing upwards are at the average values of T_0 (five scans) and the arrows pointing downwards are the average values of $T1_{/2}$.

likely that the increase in Hsp70/Hsc70 protein is primarily due to an increase in Hsp70 since we have found that *hsc70* mRNA levels are not altered in heart or other tissues when adult frogs are placed at 26°C (A. Ali and J. J. Heikkila, unpublished results). The only other published study to date examining the effect of hyperthermia (33°C for a total of 3 h) on *hsp70* gene expression in adult *Xenopus laevis* tissue found that *hsp70* mRNA accumulation was inducible to a similar level in all tissues examined, however lower temperatures or the accumulation of *hsp70* mRNA in heart tissue was not examined (Bienz 1984a). The correlation between HSF activation and *hsp70* mRNA accumulation in heart tissue following hyperthermia has also been reported in rats, whose body temperature was elevated from 37°C to 42°C (Locke et al 1995). A similar correlation has been made in rat aorta in which an increase in blood pressure resulted in HSF activation and hsp70 mRNA accumulation (Xu et al 1996). An increase in *hsp70* gene expression in response to mild hyperthermic conditions has also been reported in rainbow trout fibroblasts (Mosser et al 1986). In this latter study, Hsp70 was first heat-inducible at 26°C. However, to our knowledge, similar data examining the heat shock response in heart tissue after whole body hyperthermia in fish has not yet been reported.

These results demonstrate that a mild hyperthermic treatment can induce a preferential activation of HSF and *hsp70* gene expression in adult frog heart tissue at 26°C. Since previous studies have demonstrated that heat shock can also induce protein denaturation in cells (Lepock et al 1993), we have used DSC to compare the temperatures at which protein denaturation occurs in heart and liver tissue. Our results suggest that protein denaturation occurs at lower temperatures in heart tissue than in liver. First the onset of protein denaturation (T_0) occurs approximately 8.5°C lower in heart compared to liver. Secondly the midpoint of the DSC profile ($T_{1/2}$) is approximately 10.4°C lower in heart than in liver. Taken together these results suggest that heart proteins are generally more thermolabile than proteins in liver tissue which is consistent with previous results showing that $T_{1/2}$ of liver, muscle and lens tissue from rat rabbit and rainbow trout differ by up to 33°C (Ritchie et al 1993). Thus proteins in different tissues of the same organism and in the same tissue from different organisms have diverse thermostabilites. The DSC results are of interest given the postulated mechanism responsible for HSF activation and *hsp* gene expression. A number of studies have suggested that heat shock results in an increase in the levels of unfolded protein which in turn signal to activate HSF and *hsp* gene transcription (reviewed by Morimoto et al 1996). It is thought that the HSF monomer may be bound to Hsp70 and then released due to the heat shock-induced increase in unfolded protein. While Hsp70 binds to denatured protein, HSF converts to a trimer and binds to the HSE in heat shock genes and promotes transcription. The present results demonstrating preferential activation of HSF-binding activity and *hsp70* gene expression in adult *Xenopus* heart tissue compared to other tissues such as liver strongly support the hypothesis that activation of HSF is controlled at some level by protein denaturation.

It is likely that the enhanced accumulation of *hsp70* mRNA and protein after mild hyperthermia in heart as well as the presence of relatively abundant constitutive levels of *hsc70* mRNA (Ali et al 1996) and protein may be indicative of a protective role. A number of studies have shown in isolated and perfused heart muscle that *hsp* gene expression was associated with improved postischemic myocardial contractile recovery (Currie et al 1988; Karmazyn et al 1990; Yellon et al 1992). In fact a direct correlation between the amount of inducible Hsp70 and the degree of myocardial protection, as measured by a reduction in myocardial infarct size has been reported (Hutter et al 1994). Similarly, it has been shown that transgenic mice expressing the human *hsp70* gene have improved postischemic myocardial recovery (Plumier et al 1995). Furthermore, in *Iguana iguana* cultured heart cells, IgH-2, it was shown that a pretreatment at non-lethal temperature enables the heart cells to survive better at the high temperature which would otherwise be lethal (Bols et al 1990). Thus, it is possible that the increase in stress protein gene expression in *Xenopus* heart may have a protective function. Presumably this would occur through the role of Hsps as chaperones such that they would interact with denatured and malfolded proteins and inhibit the formation of non-functional aggregates as has been suggested for other systems (reviewed by Morimoto et al 1994).

Thus far, this discussion has been limited primarily to heart tissue but it should be noted that in the other tissues examined *hsp70* and *hsp30* mRNA were induced to varying relative levels in frogs maintained at 28°C or 30°C. For example, in frogs maintained at 28°C, *hsp30* mRNA was detected preferentially in muscle and heart while *hsp70* mRNA levels were highest in heart followed by muscle, liver and spleen. At 30°C *hsp30* mRNA accumulation was highest in muscle followed by heart, liver, eyes and spleen while for *hsp70* mRNA the highest relative level was found in heart followed by muscle, spleen, liver and eye. Thus, there appears to be a tissue-specific difference with respect to *hsp70* and *hsp30* mRNA accumulation following mild hyperthermia in adult *Xenopus*. In a previous study by Bienz (1984a) adult frogs maintained at 33°C for a total of 3 h revealed a relatively constant level of induced *hsp70* mRNA in each of the tissues examined while *hsp30* mRNA was found at relatively higher levels in kidney and gut with lower levels in liver and lung. The constant level of induced *hsp70* mRNA in the four different tissues examined may be the result of maximal transcription at this relatively high temperature for adult frogs. Also, in this latter study, lower temperatures or other organs such as heart and muscle were not examined. Tissue-specific differences with respect to heat-induced Hsp synthesis or *hsp* mRNA accumulation has also been reported in adult rats following hyperthermia (Blake et al 1990; Nowak et al 1990; Currie and White 1983). For example, Blake et al (1990) found that exposure of rats to a temperature of 35°C for 90 min resulted in the induction of *hsp70* mRNA accumulation in brain and lung but not in liver while at 37.5°C *hsp70* mRNA was detected in all of the tissues examined. While the mechanism(s) responsible for the different levels of relative *hsp70* and *hsp30* mRNA accumulation in the selected tissues of hyperthermic adult *Xenopus* is not known, it is possible that this phenomenon may reflect the specific cellular requirements for the various chaperones in different tissues.

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