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CHANGES IN SUPEROXIDE DISMUTASE AND CATALASE IN AGING HEAT-SHOCKED *DROSOPHILA*

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We have examined the age-dependent expression of CuZn superoxide dismutase (SOD) and catalase (CAT) in *Drosophila melanogaster* following heat shock. Quantitative northern blot analysis was performed after heat shock on 2-, 23- and 49-day-old flies, using a 0.48 kb SalI EcoR1 fragment of the *Drosophila* SOD cDNA and a 1.4 kb fragment of the human catalase cDNA. Heat shock induction was monitored with a 5.4 kb DNA fragment of the *Drosophila* hsp70 gene. After exposure to 37°C for 30 min and 60 min, the level of SOD RNA in young flies was elevated above that of nonstressed conditions. Changes in the transcription of SOD gene with age paralleled the expression of hsp70 RNA. The SOD RNA was elevated in heat-shocked middle aged (23–25 days old) flies compared to young *Drosophila* (2 days old), then it decreased in 49–50-day-old flies. The relative expression of CAT RNA did not change with age or after heat shock. Enzymatic activities of these two antioxidant enzymes were evaluated in nondenaturing polyacrylamide electrophoresis gels. SOD migrates on this gel as three different electromorphs. These were designated as fast, intermediate and slow migrating bands. The most intense activity was associated with the fast band in these flies. In the absence of heat shock, there was an age-dependent decrease in the intermediate, but not in the slow or fast bands. Heat shock does not affect the intensity of the fast band in young or old flies, however; in middle aged flies, there is a shift in this band toward the slow position. No change was detected in the activity of catalase with age or heat shock, although flies of all ages exhibited a shift toward a faster-migrating electromorph with increasing time of heat shock. This effect was also observed in flies fed H_2O_2 and is more pronounced in insects fed higher concentrations. These results are discussed in relation to the role of these antioxidant enzymes in protecting against age-induced oxidative stresses.

KEY WORDS: Superoxide dismutase, catalase, hsp70, heat shock, aging, *Drosophila melanogaster*

INTRODUCTION

Cells continuously exposed to either exogenous or endogenous stress such as heat, oxygen radicals or radiation have developed a variety of protective mechanisms, such as the synthesis of stress proteins, antioxidant defense or DNA repair enzymes. The similarity of responses to elevated temperatures, and various oxidative stresses in bacteria,[1–4] *Drosophila*[5] and even in plants[6] suggest the possibility that increased temperature and oxidative stress may be related. Accordingly, cells or organisms exposed to heat or oxidative stress synthesize a similar subset of "heat shock proteins".

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The induction of these proteins is necessary to survive a given stress and recover to normal metabolic conditions.

Exposure to heat is accompanied by an elevation in oxygen consumption. Thus, exposure to high temperatures most likely results in an increased production of free radicals which would generate the need for an elevated level of scavenging enzymes to remove these toxic species. Consequently, enzymes such as superoxide dismutase (SOD) and catalase (CAT) which defend cellular components against oxidative damage may contribute to the cellular defense system during heat stress. SOD, which is the primary defense against intracellular superoxide, exists in two forms in eukaryotic cells: as a Cu/Zn SOD which resides in the cytosol and also may reside in the peroxisomes and a Mn SOD which is localized in the mitochondria. The cytoplasmic form of SOD is a 32 kDa dimer with two Cu and two Zn atoms and catalyzes the dismutation of the superoxide anion ($O_2^{\cdot-}$) to hydrogen peroxide and molecular oxygen.[7,8] The product of the dismutation reaction, H_2O_2, is removed by catalase, a 240 kDa tetramer, in peroxisomes and by glutathione peroxidase in other cell compartments.

Though an effective antioxidant-scavenging system is necessary during normal metabolic conditions to protect cellular components against oxidative damage, the need for this protection increases during periods of stress, such as exposure to heat. Experimental evidence suggests that if heat-induced oxidative stress exceeds the antioxidant defense capability of the cell, this can result in a heat-induced injury and lead to cell death. Accordingly, it has been shown that normal mouse embryo cells with 2–5 times higher level of SOD, CAT and glutathione peroxidase than SV-40 transformed cells are more resistant to hyperthermia (cell survival 75% vs. 5%).[9] Pretreatment of normal cells with diethyldithiocarbamate (the inhibitor of SOD activity) significantly reduced this resistance and in the case of transformed cells made them extremely thermosensitive.[9] Also, in several other systems, an increased antioxidant level has been correlated with thermal tolerance.[10,11]

The extent of oxidative damage to biological molecules has been suggested to play a major role in the aging process.[12] Numerous studies have shown that during normal cellular aging the level of oxidative stress (i.e. balance between prooxidants and antioxidants) shifts toward a relatively more prooxidizing status.[13] This may result in the increase in oxidative damage to cellular components with age[14] and consequently reduce the metabolic efficiency of a cell. Accordingly, a decrease in enzymatic activity of the scavenging enzymes SOD, CAT, and also glutathione was observed in house flies with age.[15] Also SOD and CAT expression was lower in the brain and liver of aging rats.[16] However, the literature contains conflicting data in this regard and the analysis of antioxidant enzymes in some systems indicates that there is no significant decrease in antioxidant defense with age.[17–19]

The extent of cellular damage may be especially relevant in senescent organisms during adaptation to environmental stresses such as heat shock. We have previously shown that old *Drosophila* are less resistant to heat than young flies, and we have postulated that this may be related to an increase in age-dependent protein damage.[20] There are no data describing the heat-dependent regulation of antioxidant genes in aging organisms. In this study we have investigated the expression of SOD and CAT in aging *Drosophila* during exposure to heat shock. These results have important implications for evaluating mechanisms that protect organisms against heat stress during aging.

MATERIALS AND METHODS

Drosophila Cultures and Heat Shock

Drosophila melanogaster of the Oregon R strain, obtained in 1981 from NASA Ames Research Center, were maintained at 24°C in half-pint bottles containing Formula 4-24 instant *Drosophila* medium (Carolina Biological Supply Co.).

Heat shock was applied by transferring 20 male flies of given age group to 35 ml thin-walled glass vials (containing filter paper moistened with 25% sucrose) and placing them in a 37°C incubator for different periods of time.

Isolation of RNA

Total RNA was prepared by placing 20 flies in a 1.5 ml microcentrifuge tube and homogenizing in a solution containing equal volumes of phenol and buffer (10 mM Tris-HCl pH 7.5, 20 mM EDTA, 1% SDS) as described by Xiao and Lis.[21] The RNA was sequentially extracted by phenol, phenol/chloroform, and chloroform; collected by ethanol precipitation, and suspended in H_2O. Samples were kept frozen at −70°C until use. The concentration of RNA was determined by taking an absorbance reading at 260 and 280 nm, assuming that 1 mg of RNA/ml of solution gives 25 A units at 260 nm.[22]

Analysis of RNA by Northern Blotting

Total RNA (4 ug/lane) was size fractionated on 0.9% formaldehyde-agarose gels according to the method described by Maniatis.[22] RNA was transferred to a piece of Biotrans nylon membrane. The membrane was baked for 2 h at 80°C *in vacuo* and prehybridized for 3 h at 45°C in a prehybridization mixture containing 50% (v/v) formamide, 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, 25 ug/ml sonicated sperm DNA. The membrane was probed with denatured ^{32}P-labeled 0.48 kb *Sal*1/*Eco*R1 fragment of the pGem2 containing the *Drosophila* Cu/Zn SOD (gift from J. Kwiatowski, UC Irvine, CA) gene or with 1.4 kb fragment of *Eco*R1/*Sal*1 human catalase gene (obtained from R. Hallewell, Chiron Corp., Emeryville, CA).[23] Hybridization was performed at 45°C overnight. The membrane was washed for 1 h at room temp. in 2 × SSC, 0.1% SDS and then for 1 h at 50°C in 0.1 × SSC, 0.5% SDS and finally rinsed in 0.1 × SSC. The membrane was exposed to Kodak XRP-1 x-ray film with an intensifying screen at −70°C for different times. The filters were stripped of the label and rehybridized under the same conditions (except the wash in 0.1 × SSC, 0.5% SDS was at 58°C) with a 5.4 kb *Sal*1 fragment of the p132E3 plasmid of the *Drosophila* hsp70 gene (gift from S. Munroe, Stanford University, CA) and 0.8 kb *Sal*1 fragment of *Drosophila* actin 42A gene (gift from B.B. Mathews, UCLA, CA).

SOD Activity

Extraction of Cu/Zn SOD from flies was performed by the method of Bannister and Bannister,[24] modified as follows: 25 flies were homogenized in 150 ul of 20 mM Tris-acetate buffer pH 7.8 containing 0.2% Triton X-100 and 1 mM phenylmethyl-

sulfonyl fluoride. The homogenate was centrifuged at 11,000 × g for 15 min at 4°C. The resulting supernatant was collected, heated rapidly to 60°C for 10 min, acidified to pH 5.5 with 1 M acetic acid, and centrifuged at 11,000 × g for 15 min at 4°C. Proteins were separated on a 10% nondenaturing polyacrylamide gel for 18 h in 35 V. After electrophoresis, SODs were identified in the gel by the Nitro Blue Tetrazolium method.[25] The gels were soaked in 2.5 mM Nitro Blue Tetrazolium for 30 min with shaking. They were rinsed and immersed in a solution of 0.028 M tetramethylene diamine, 2.8 × 10 − 5 M riboflavin and 0.036 M potassium phosphate at pH 7.8. The gels were then illuminated on a fluorescent light box for 5 to 15 min. During that time, gels gradually become uniformly blue (reduced Nitro Blue Tetrazolium) except at the location of SOD.

Catalase Activity

Protein crude extracts were prepared from *Drosophila* of different age groups. 25 males were homogenized in 250 ul of 20 mM Tris-Acetate buffer pH 7.8, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 11,000 × g for 15 min at 4°C and the collected supernatant was used for protein analysis. Crude extracts were loaded on a 8% polyacrylamide nondenaturing gel and the proteins were separated overnight at 55 V. After electrophoresis, the CAT bands were identified in the gel using a modification of the method of Gregory and Fridovich.[26] Gels were soaked for 15 min in a 1 to 1000 dilution of 3% H_2O_2. Then gels were immersed in freshly prepared solution of 0.2% potassium ferricyanide, 0.2% ferric chloride and mixed gently for a few minutes. Catalase activity appears as yellow zones on a blue-green background.

RESULTS

We have analysed the expression of SOD and CAT RNA in male *Drosophila* 2 days, 23–24 days and 49–50 days old. Northern blots were performed from total RNA isolated from these flies kept at normal environmental temperature (25°C) and heat shocked at 37°C for 30 min and 60 min. Blots were probed with 0.48 kb fragment of *Drosophila* SOD cDNA and 1.4 kb fragment of human catalase cDNA. Blots were also probed with 5.4 kb DNA fragment containing *Drosophila* hsp70 gene which served to monitor the induction of the heat shock response (Figure 1). To correct for differences in gel-loading transfer or RNA degradation, the amount of hsp70, CAT and Cu/Zn SOD was determined as a ratio to actin (Table I). This was accomplished by rehybridization of filters with 0.8 kb *Sal* I fragment of *Drosophila* actin 42A gene. Blots for each particular probe were exposed for different times to obtain the optimal density of bands for densitometric scanning of autoradiographs. As can be seen in both Figure 1 and Table I, the exposure of flies of different ages to 37°C results in the induction of 2.3 kb RNA corresponding to the full size hsp70 RNA transcript. In RNA samples isolated from flies kept at 25°C, no hybridization signal with hsp70 probe was detected. Data in Table I show that hsp70 RNA synthesis was higher after 60 min than after 30 min of heat treatment. This is in agreement with the results reported from Craig's laboratory on adult *Drosophila*[27] as well as with reports showing that an increase in hsp70 is correlated with the severity of heat

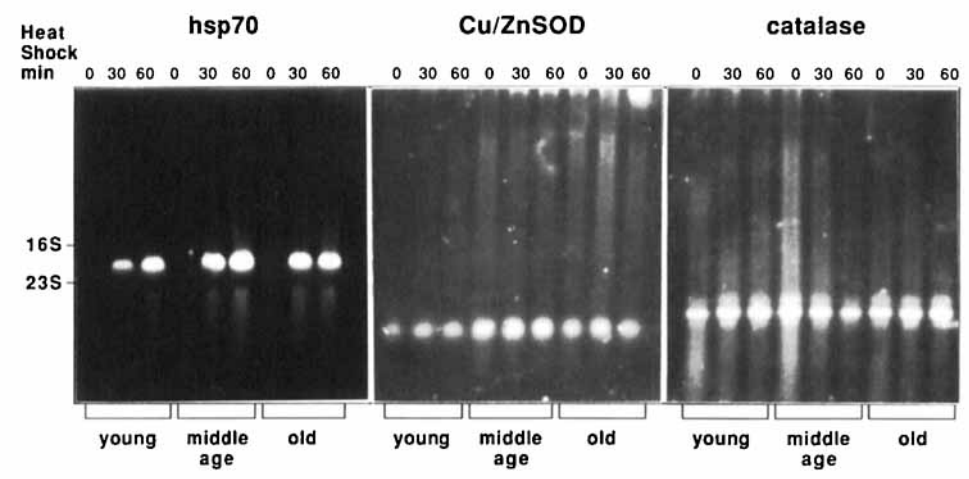

FIGURE 1 Changes in the expression of hsp70, Cu/Zn SOD and catalase in heat-stressed aging flies. Northern blot was performed on RNA isolated from 2-day-old (young), 23–24-day-old (middle aged) and 49–50-day-old (old) flies. Hybridization was done with 0.48 kb *Drosophila* Cu/Zn SOD, 1.4 kb human catalase and 5.4 kb *Drosophila* hsp70 gene as described in Materials and Methods.

TABLE I
Effect of age and heat stress on the expression of Hsp 70, Cu/ZnSOD and CAT mRNA in *Drosophila*

	Time of heat shock (min)								
	Hsp 70 actin			Cu/ZnSOD actin			CAT actin		
Age	0	30	60	0	30	60	0	30	60
2 days old	0	1.4	2.6	0.8	1.1	1.2	0.35	0.35	0.38
23–24 days old	0	2.8	3.9	2.8	3.4	3.5	0.40	0.40	0.33
49–50 days old	0	2.0	3.4	1.6	2.5	1.8	0.36	0.38	0.40

Ratios of hsp70, Cu/ZnSOD and CAT mRNA to actin were calculated from the integration data of the hybridization bands as described in Materials and Methods. Data presented are the average of two measurements.

stress in cultured Schneider cells.[28] According to our earlier report,[20] hsp70 synthesis was highest in middle aged flies compared to young and old *Drosophila.*

Total RNA isolated from control and heat-shocked flies of different ages was rehybridized with 0.48 kb fragment of *Drosophila* Cu/Zn SOD cDNA. As can be seen on Figure 1, a band corresponding the *Drosophila* Cu/Zn SOD transcript hybridizes to the probe in flies of all ages kept both at 37°C and at their normal environmental temperature of 25°C. Following exposure to 37°C for 30 min and 60 min, the level of SOD RNA was elevated above that observed under non-stressed conditions in 2-day-old insects (Table I). However, it did not increase further with time of heat shock. We did not observe significant changes in SOD expression by heat shock in flies of other ages. No significant differences in the level of SOD RNA

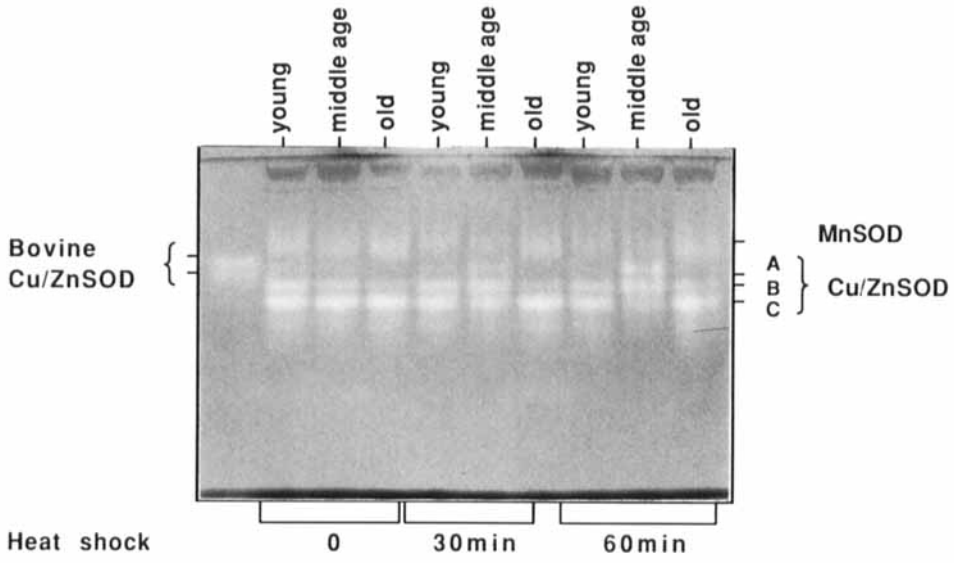

FIGURE 2 Polyacrylamide gel analysis of SOD activity in heat-shocked aging flies. The positions of MnSOD and three different Cu/Zn SOD electromorphs (A, B, C) are shown in young (4 days old), middle aged (23–24 days old) and old (50 days old) control flies and flies heat-shocked for 30 and 60 min. Purified bovine Cu/Zn SOD was coelectrophoresed as a standard.

were detected between 30 and 60 min of heat shock for flies that are young (2 days old) or middle aged (23–24 days old). In the case of 49–50-day-old insects, a decrease in SOD expression was noted after 60 min (compared to 30 min) of heat treatment. The SOD RNA level in flies kept at normal environmental temperature was 3.5 times elevated in 23–24-day-old flies compared to 2-day-old insects, then decreased in old flies. However, old flies still expressed 2 times more SOD than the young ones in the absence of heat shock. The same age-dependent pattern was also observed in heat-stressed flies and parallels the changes for hsp70 RNA expression with age (Table I).

Figure 1 also shows a northern blot analysis of CAT RNA in flies of different ages exposed for 30 min and 60 min and kept at 25°C. Calculated CAT/actin ratios show that CAT RNA level does not significantly change in flies both with age and after heat treatment for either 30 min and 60 min (Table I).

Changes in enzymatic activity of SOD and CAT in flies of different ages exposed to heat stress were analyzed by polyacrylamide gel analysis. SOD polyacrylamide activity gel is shown in Figure 2 and densitometric profiles from this gel are presented in Figure 3, for young, middle aged and old flies kept at 25°C and heat-shocked for 30 min and 60 min at 37°C. The active Cu/Zn SOD enzyme is a homodimer composed of two 16-kDa subunits. On the activity gel, 3 bands corresponding to different electromorphs of *Drosophila* Cu/Zn SOD were detected (A, B, C). Among these electromorphs the strongest activity was associated with the fastest migrating SOD band on this gel. The upper, slowest-migrating band on the gel corresponds to the position of *Drosophila* Mn SOD. We observed an age-dependent small decrease in the intensity of the intermediate Cu/Zn SOD electromorph band (B) in flies not

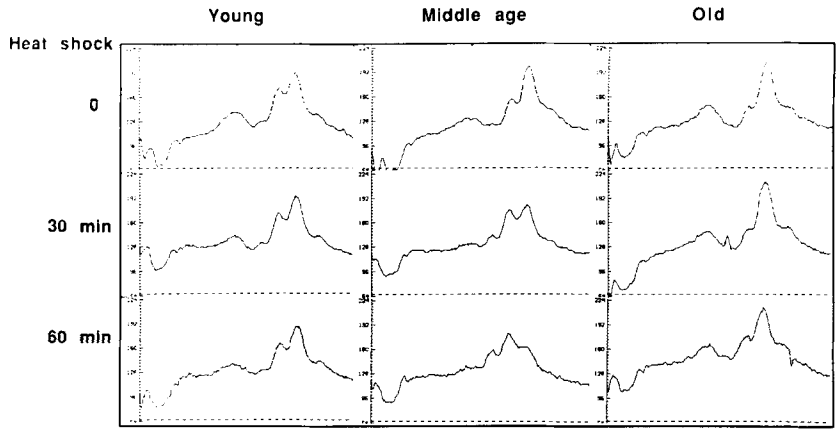

FIGURE 3 Densitometric profiles of SOD activity on polyacrylamide gels. Changes in migration position of three different Cu/Zn SOD electromorphs are shown for young (4 days old), middle aged (23–24 days old) and old (50 days old) control flies and flies exposed to heat stress for 30 and 60 min. The direction of band migration is left to right.

exposed to heat stress (see Figure 3). The intensity of the fast electromorph (C) was not affected by age. Exposure of flies to 37°C for 30 min and 60 min does not significantly change the intensity of the faster-migrating band (C) in young and old *Drosophila* on the activity gel. However in middle aged flies, there is a shift in the position of this SOD band toward slowest-migrating electromorph (A) at the expense of the fastest one (C). This pattern in the shift in Cu/Zn SOD activity is very clear in middle aged flies after 60 min of heat exposure (Figure 3). The intensity of the Mn SOD band on the activity gel shows a tendency to decrease slightly during heat-shock treatment compared to nonstressed conditions in flies of all ages studied.

Polyacrylamide gel analysis and densitometric profiles of CAT activity in flies of different ages exposed to 30 min and 60 min of heat stress is presented on Figures 4 and 5, respectively. There is no evident change in CAT activity in flies with age both during normal and heat-shock conditions. However, flies of all ages exhibit a shift toward fastest-migrating electromorph with the increasing time of heat shock (Figure 5). The tendency to this change is observed earlier during heat stress in old than in young flies. For instance, the activity shift can be seen in old flies after 30 min of heat exposure, but not in young and middle aged organisms, where it is evident after 60 min of heat stress.

It has been reported that aging flies have increased level of H_2O_2 released from mitochondria as a result of mitochondrial membrane damage and/or increased intensity of oxidative stress with age.[29,30] Thus, we addressed the question of how the exposure of *Drosophila* to an increased level of H_2O_2 affects CAT activity. We fed young *Drosophila* on 1% and 3% H_2O_2 for 48 hours and analyzed CAT activity by polyacrylamide gel electrophoresis. The results of this assay are shown on Figure 6. Flies fed on sucrose display one band of CAT activity on the gel. In flies exposed to an increasing concentration of H_2O_2 for 24 hrs, there is a shift in the CAT activity band toward faster-migrating position. The shift toward faster-migrating band is more pronounced in CAT isolated from flies exposed to a H_2O_2 concentration of 3% than of 1%.

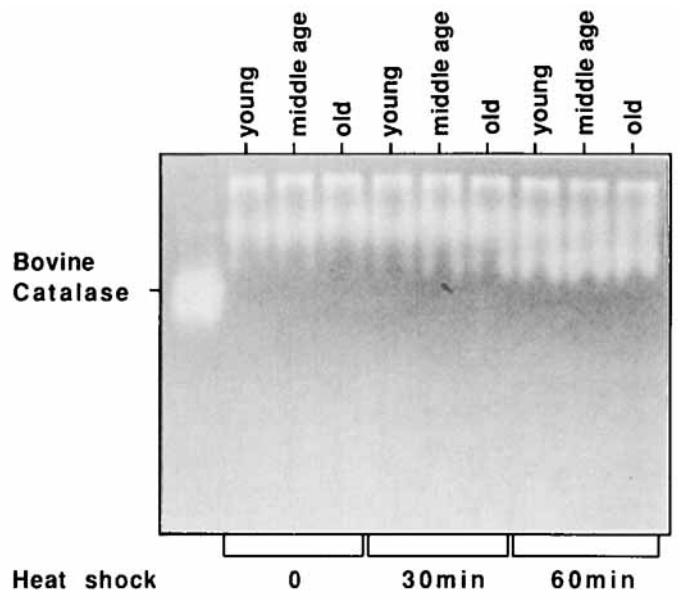

FIGURE 4 Polyacrylamide gel analysis of catalase activity in aging heat-shocked flies. Catalase activity was determined as described in Materials and Methods. Purified bovine catalase was used as a standard.

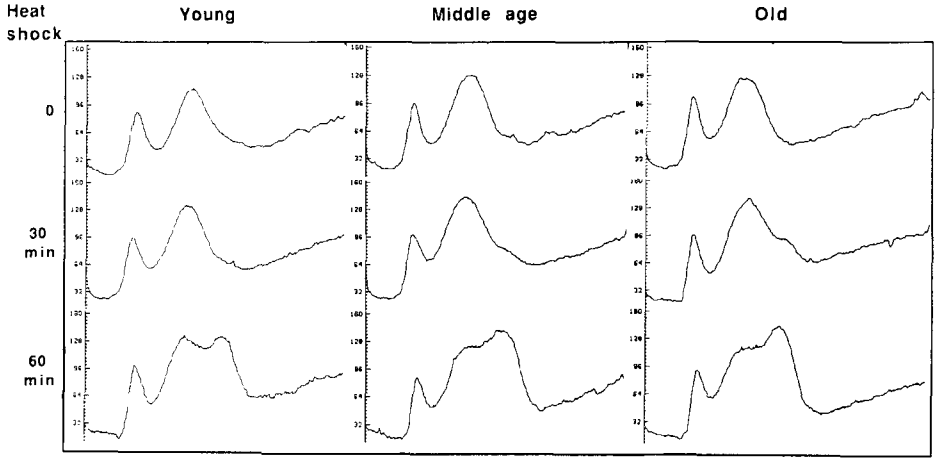

FIGURE 5 Densitometric profiles of catalase activity from polyacrylamide gels. The migration position of catalase activity is shown in young (4 days old) middle aged (23–24 days old) and old (50 days old) flies non-stressed and exposed to 30 and 60 min of heat stress. The direction of band migration is left to right.

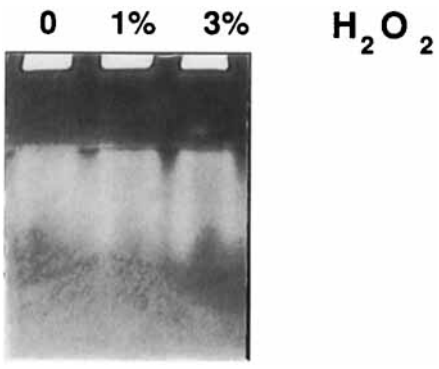

FIGURE 6 Polyacrylamide gel analysis of catalase activity in young flies exposed to 1% and 3% of H_2O_2.

DISCUSSION

Because SOD and CAT play an important role in free radical detoxification, the age-related changes in the expression of these two enzymes during exposure to stress may predispose organisms to free-radical damage.

In our studies we analyzed how exposure to heat shock affects the transcription and enzymatic activity of Cu/Zn SOD and CAT and whether these changes are age-related.

We observed that the expression of Cu/Zn SOD and CAT were differently affected by elevated temperature in aging *Drosophila*. The level of *Drosophila* Cu/Zn SOD RNA changed with both age and heat stress. Exposure of young flies to 30 min and 60 min of heat stimulated expression of *Drosophila* SOD, however, in middle aged and old flies SOD RNA level was not much affected compared to nonstressed conditions. The induction of Cu/Zn SOD by heat shock was observed in mammalian cells,[31] also bacterial SOD was elevated by heat exposure.[4]

It is interesting that Cu/Zn SOD RNA synthesis in aging heat stressed flies parallels the age related changes in hsp70 expression under these conditions. The maximum expression for both Cu/Zn SOD and hsp70 was observed during heat-stress in middle aged (23–24-day-old) flies. For instance, old flies (50 days old) exposed to 30 min of heat treatment had about 22% less RNA for hsp70 and 26% for SOD compared to middle aged insects. However, old flies still had about 1.2 times more hsp70 RNA and 2.3 times more Cu/Zn SOD RNA compared to 2-day-old *Drosophila*. On the other hand, while hsp70 RNA synthesis increased with time of heat shock, the Cu/Zn SOD RNA level in flies of different ages did not change significantly between 30 and 60 min of heat exposure.

Thermodynamic analysis shows that the primary targets of heat-induced damage are proteins.[32] Moreover, oxygen radicals have been shown to induce posttranslational damage to polypeptides *in vivo*.[33,34] The synthesis of hsp70 is necessary for the protection of cellular proteins against heat-induced denaturation, and the extent of its expression has been correlated with the severity of cellular damage. According to this, an elevated level of hsp70 was observed in heat-stressed middle aged flies relative to young flies. However, in old *Drosophila*, where even higher accumulation of abnormal proteins is expected, the hsp70 synthesis is lower

than in middle aged organisms. The decrease in hsp70 synthesis in heat-stressed old compared to middle aged flies is also accompanied by lower Cu/Zn SOD expression. This may result in more cellular protein damage generated during heat exposure in flies of this age. Thus, the coordinate expression of Cu/Zn SOD and hsp70 during heat stress with age suggest that these proteins may be a part of a protective system that is induced by heat stress and similarly affected by age. The indirect protective effect of Cu/Zn SOD against protein oxidation may be involved in other cellular events in addition to heat stress, such as the resistance to TNF,[35] and to viral infection.[36] In all of these cases, both the increase in the level of abnormal cellular proteins and oxidative damage seem to play a role.

Induction of Cu/Zn SOD by heat in young flies and its age related coordinate expression with hsp70 would also suggest that Cu/Zn SOD may play a function of stress protein. However, the published data do not show the presence of the Heat Shock consensus sequence in the promoter region of the *Drosophila* Cu/Zn SOD gene which is necessary for heat induction. It is likely that different types of regulation may also be involved in the transcriptional stimulation of *Drosophila* Cu/Zn SOD gene during heat stress and they may undergo age related changes. Examples of such regulation have been found in yeast CAT[37] and also in *S. cerevisiae* DDR2 gene, which can be activated by both heat and DNA damage without Heat Shock Element involved.[40]

Heat-induced transcription of *Drosophila* Cu/Zn SOD does not directly correlate with the changes in Cu/Zn SOD enzymatic activity in aging flies during heat shock. The enzymatic activity of Cu/Zn SOD is affected by heat shock only in middle aged flies. In young and old heat stressed flies, the relative intensities of the Cu/Zn SOD electromorphs are not substantially different from nonstressed *Drosophila* of corresponding ages. This lack of correlation between heat-induced increase in transcription of Cu/Zn SOD and lack of changes in its enzymatic activity may be related to the findings reported for Cu/Zn SOD in lungs of aging rats. Despite an age-related increase in Cu/Zn SOD transcript, total Cu/Zn SOD enzymatic activity remained constant due to an increase in the relative proportion of inactive Cu/Zn SOD in this aging species.[39] In middle aged flies, we observed maximum expression of Cu/Zn SOD RNA during heat stress and it is interesting that only at this age Cu/Zn SOD activity was affected by heat. This change in activity was manifested by a shift in the position of the fast and slow electromorph on the nondenaturing gel. Our activity gel shows that the various Cu/Zn SOD electromorphs have different scavenging activities. It has been also observed that there is a selection for one of these electromorphs in long-lived *Drosophila* strain (M. Rose, personal communication).

In flies of all ages, CAT RNA level was not changed during heat treatment compared to nonstressed conditions. The similar lack of induction of CAT was also observed for rat hearts during heat shock.[40] However, it has been reported that heat stress induced CAT RNA in yeast[37] and Neurospora.[41]

It is interesting that the expression of catalase RNA is not affected by heat stress, whereas the expression of the Cu/Zn SOD transcript increases following heat shock. Cu/Zn SOD is an exclusive $O_2^{\cdot-}$ scavenger, while H_2O_2, the product of the Cu/Zn SOD dismutation reaction, is converted to H_2O and O_2 not only by CAT but also other scavengers such as GSH, ascorbate and glutathione peroxidase.[42] The efficiency of H_2O_2 scavengers other than CAT may partially explain the lack of change in the transcription rate of the CAT gene during heat stress. This is supported by the

observation that changes in cellular GSH level following heat stress in CHO cells are correlated with the thermal sensitivity.[11] Our results show that despite lack of induction of transcription heat shock alters CAT enzymatic activity. We observed a shift in the migration of CAT activity band on nondenaturing polyacrylamide gel toward a faster migrating position. The shift in CAT activity band is related to the intensity of the heat stress (it is more pronounced after 60 min than 30 min of heat treatment) (Figures 4 and 5).

We have observed that exposure of young flies at normal physiological temperature to increased concentration of H_2O_2 (1% to 3%) resulted in a shift in CAT activity similar to the one observed in *Drosophila* exposed to heat stress. Sohal[30] reported an increasing release of H_2O_2 from mitochondria with age, but there are no data regarding any concomitant physicochemical change of the CAT protein. In aging *Drosophila* maintained at physiological temperature, we did not observe any change in the position of the CAT band on non-denaturing activity gel. However, during heat stress, the shift of CAT activity toward faster-migrating position was noted and it was observed earlier in senescent than in young *Drosophila* (Figure 5, compare 30 min heat shock for young, middle aged and old flies). This would suggest the existence of a threshold in the cellular H_2O_2 level affecting CAT protein resulting in a change in the migration of this protein on non-denaturing gel.

Our results conclude that in aging *Drosophila*, heat stress affects both transcription of Cu/Zn SOD and CAT and their enzymatic activity but in a different, not necessarily coordinate, manner. In young flies Cu/Zn SOD transcription was stimulated by heat treatment. Changes in the transcription of the Cu/Zn SOD gene with age paralleled the expression of hsp70 RNA. After heat shock, the level of RNA for these two proteins increases in middle aged flies. The maximum expression of Cu/Zn SOD in middle aged flies correlated with changes in the distribution of different Cu/Zn SOD electromorphs at this age.

The expression of CAT was not affected by either heat and age. However, during heat exposure, CAT showed a shift toward the fastest electromorph, which may be related to the accumulation of H_2O_2 in cells and may reflect differential effects of oxidative stress in flies of different age.

References

1. B.R. Bonner, P.C. Lee, S.W. Wilson, C.W. Cutter and B.N. Ames (1984) AppppA and related adenylated nucleotides are synthesized as a consequence of oxidative stress. *Cell*, **37**, 225–232.
2. M.T. Christman, R. Morgan, F.S. Jacobsen and B.N. Ames (1985) Positive control at a regular for defenses against oxidative stress and some heat shock proteins in *Salmonella typhimurium*. *Cell*, **41**, 753–762.
3. R.A. Van Bogelen, P.M. Kelley and F.C. Neidhart (1987) Differential induction of heat shock, SOD, and oxidative stress regulons and accumulation of nucleotides in *Escherichia coli*. *Journal of Bacteriology*, **169**, 26–32.
4. C.T. Privale and I. Fridovich (1987) Induction of superoxide dismutase in *Escherichia coli* by heat shock. *Proceedings of the National Academy of Sciences USA*, **84**, 2723–2726.
5. M. Ashburner and J.J. Bonner (1979) The induction of gene activity in *Drosophila* by heat shock. *Cell*, **17**, 241–254.
6. E. Czarnecka, L. Edelman, F. Schaffl and J.L. Key (1984) Comparative analysis of physical stress responses in soybean seedlings using cloned heat shock cDNAs. *Plant Molecular Biology*, **3**, 45–58.
7. J.M. McCord, J.A. Boyle, D. Day, L. Rizzolo and M.L. Salin (1977) A manganese-containing superoxide dismutase from human liver. In *Superoxide and Superoxide Dismutases* (eds. A.M. Michelson, J.M. McCord and I. Fridovich). Academic Press, London and New York, pp. 129–138.
8. J.M. McCord and I. Fridovich (1969) Superoxide dismutase: An enzymatic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, **224**, 6049–6055.

9. R.A. Omar, S. Yano and Y. Kikkawa (1987) Antioxidant enzymes and survival of normal and simian virus 40-transformed mouse embryo cells after hyperthermia. *Cancer Research*, **47**, 3473–3476.
10. S.L. Pardue, J.P. Thaxton and J. Brake (1985) Role of ascorbic acid in chicks exposed to high environmental temperature. *Journal of Applied Physiology*, **58**, 1511–1516.
11. M.L. Freeman and M.J. Meredith (1988) Subcellular localization of glutathione and thermal sensitivity. *Radiation Research*, **115**, 461–471.
12. D. Harman (1956) Aging: A theory based on free radicals and radiation chemistry. *Journal of Gerontology*, **11**, 298–300.
13. R.S. Sohal, L.A. Arnold and B.H. Sohal (1990) Age-related changes in antixoidant and prooxidant generation in tissues of the rat with special reference to parameters in the insect species. *Free Radical Biology and Medicine*, **10**, 495–500.
14. J. Miquel and J.E. Fleming (1986) Theoretical and experimental support for an oxygen-radical-mitochondrial injury hypothesis of cell aging. In *Free Radicals, Aging and Degenerative Diseases* (eds. J.E. Johnson, R. Willford, D. Harman and J. Miquel). Alan R. Lis, New York, pp. 57–74.
15. R.J. Sohal, K.J. Farmer, R.G. Allen and N.R. Cohen (1983) Effect of age on oxygen consumption, superoxide dismutase, catalase, glutathione, inorganic peroxides and chloroform-soluble antioxidant in the adult male housefly, *Musca Domestica*. *Mechanisms of Ageing and Development*, **24**, 185–195.
16. I. Semsei, G. Rao and A. Richardson (1991) Expression of superoxide dismutase and catalase in rat brain as a function of age. *Mechanisms of Ageing and Development*, **58**, 13–19.
17. R. Perez-Campo, M. Lopez-Torres, D. Paton, E. Sequeros and G. Barja de Quiroga (1990) Lung antioxidant enzymes, peroxidation, glutathione system and oxygen consumption in catalase inactivated young and old *Rana perezi* frogs. *Mechanisms of Ageing and Development*, **56**, 281–292.
18. U. Reiss and D. Gershon (1976) Comparison of cytoplasmic superoxide dismutase in liver, heart and brain of aging rats and mice. *Biochemical and Biophysical Research Communications*, **73**, 255–262.
19. G. Barja de Quiroga, R. Pérez-Campo and M. López Torres (1990) Antioxidant defences and peroxidation in liver and brain of aged rats. *Biochemical Journal*, **272**, 247–250.
20. A. Niedzwiecki, A.M. Kongpachith and J.E. Fleming (1991) Aging affects expression of 70-kDa heat shock proteins in *Drosophila*. *Journal of Biological Chemistry*, **266**, 9332–9338.
21. H. Xiao and J.T. Lis (1989) Heat shock and developmental regulation of the *Drosophila melanogaster* hsp83 gene. *Molecular and Cellular Biology*, **9**, 1746–1753.
22. T. Maniatis, E.F. Fritsch and J. Sambrook (1985) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. A.P. Feinberg and B. Vogelstein (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Chemistry*, **132**, 6–13.
24. J. Bannister and W. Bannister (1985) Purification of copper/zinc superoxide dismutase. In *Handbook of Methods for Oxygen Radicals Research* (ed. R.A. Greenwald). CRC Press, Boca Raton, FL, pp. 5–7.
25. C. Beauchamp and I. Fridovich (1971) Superoxide dismutase: Improved assays and an assay applicable to polyacrylamide gels. *Analytical Chemistry*, **44**, 276–287.
26. A. Clairborne (1985) Catalase activity. In *CRC Handbook of Methods for Oxygen Radical Research* (ed. R.A. Greenwald). CRC Press, Boca Raton, Florida, pp. 283–284.
27. K.B. Palter, M. Watanabe, L. Stinson, A. Mahowald and E.A. Craig (1986) Expression and localization of *Drosophila melanogaster* hsp70 cognate proteins. *Molecular and Cellular Biology*, **6**, 1187–1203.
28. B.J. DiDomenico, G.E. Bugaisky and S. Lindquist (1982) The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. *Cell*, 31, 593–603.
29. J. Miquel and J.E. Fleming (1986) Theoretical and experimental support for an oxygen radical mitochondrial injury. In *Hypothesis of Cell Aging, Free Radical Aging and Degenerative Diseases* (eds. D. Harmon, J. Miquel, R. Walford and J.E. Johnson Jr.). Alan Lis, New York, pp. 51–74.
30. R.S. Sohal and B.H. Sohal (1991) Hydrogen peroxide release by mitochondria increases during aging. *Mechanisms of Ageing and Development*, **57**, 187–203.
31. M.A. Hass and D. Massaro (1988) Regulation of the synthesis of superoxide dismutases in rat lungs during oxidant and hyperthermic stresses. *Journal of Biological Chemistry*, **263**, 776–781.
32. M.F. Dubois, A.G. Hovanessian and O. Bensaude (1991) Heat-shock-induced denaturation of proteins. Characterization of the insolubilization of the interverion-induced p68 kinase. *Journal of Biological Chemistry*, **266**, 9709–9711.
33. L. Fucci, C.N. Oliver, M.J. Coon and E.R. Stadtman (1983) Inactivation of key metabolic enzymes by mixed-function oxidation reactions: Possible implications in protein turnover and ageing. *Proceedings of the National Academy of Sciences USA*, **80**, 1521–1525.
34. K.J.A. Davies (1987) Protein damage and degradation by oxygen radicals. 1. General aspects. *Journal of Biological Chemistry*, **262**, 9895–9901.

35. G.H.W. Wong, J.H. Elwell, L.W. Oberley and D.V. Goeddel (1989) Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell*, **58**, 923–931.
36. B. Phillips, K. Abravaya and R.I. Morimoto (1991) Analysis of the specificity and mechanism of transcriptional activation of the human hsp70 gene during infection by DNA viruses. *Journal of Virology*, **65**, 5680–5692.
37. R. Wieser, G. Adam, A. Wagner, C. Schüller, G. Marchler, H. Ruis, Z. Krawiec and T. Bilinski (1991) Heat shock factor-independent heat control of transcription of the *CTT1* gene encoding the cytosolic catalase T of *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, **266**, 12406–12411.
38. N. Kobayashi and K. McEntee (1990) Evidence of heat shock transcription factor-independent mechanism for heat shock induction of transcription in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences USA*, **87**, 6550–6554.
39. H. Ischiropoulos, C.E. Nadziejko and Y. Kikkawa (1990) Effect of aging on pulmonary superoxide dismutase. *Mechanisms of Ageing and Development*, **52**, 11–26.
40. R.W. Currie and R.M. Tanguay (1991) Analysis of RNA for transcripts for catalase and SP71 in rat hearts after *in vivo* hyperthermia. *Biochemistry and Cell Biology*, **69**, 375–382.
41. P. Chary and D.O. Natvig (1989) Evidence for three differentially regulated catalase genes in *Neurospora crassa*: Effects of oxidative stress, heat shock, and development. *Journal of Bacteriology*, **171**, 2646–2652.
42. A.B. Fisher (1987) Intracellular production of oxygen-derived free radicals. *Upjohn Symposium/ Oxygen Radicals*, April, pp. 34–39.