The Role of Methionine Sulfoxide Reductase in Thermal Stress Response

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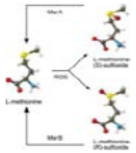
Abstract

Methionine sulfoxide reductase (Msr) is an enzyme that repairs oxidative damage to methionine and exists in two distinct, stereospecific forms: MsrA and MsrB. Hyperthermia has been observed to promote cell oxidative damage. **In this study we examined whether Msr plays a role in tolerance to hyperthermia using *Drosophila melanogaster*.** Preliminary studies have demonstrated that organisms lacking both MsrA and MsrB are less efficient in their thermal stress response when compared to flies containing MsrA and MsrB. **Given that heat shock proteins (HSPs) have also been found to counter the effects of hyperthermia, we tested if hyperthermia preconditioning improves the organism's response to thermal stress.** Future studies include examining the biochemical mechanisms that governs the effect of Msr on hyperthermia tolerance. Determining the roles of MsrA and MsrB in hyperthermia tolerance should lend insight into the reductive capabilities of MsrA and MsrB.

Introduction

Hyperthermia
- Exposure to high temperatures (1)
- Tolerance varies:
  - Mammals experience stroke
  - Invertebrates (ex. Drosophila) experience spreading depression (2)
- Induces formation of reactive oxygen species (ROS): free radicals (3)
- Heat shock proteins (HSPs) are molecular chaperones that are upregulated and provide neuronal protection during hyperthermia (5)

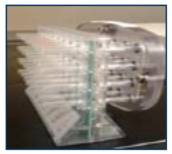
Methionine Sulfoxide Reductase
- ROS oxidize methionine to methionine sulfoxide (met-S-(o) and met-R-(o))
- Methionine Sulfoxide Reductase (Msr) reduces met – (o) into methionine
- Two stereospecific forms of Msr: MsrA (met-S-(o)) and MsrB (met-R-(o)) (6)



Hyperthermia Preconditioning
- Exposure to sub-lethal hyperthermic conditions prior to exposure to heat stress
- Heat shock proteins (HSP 70) upregulated
- Protects organisms from the effects of thermal stress (7)

Materials and Methods

DAM system:
- Measures the movement of the flies every minute via an infrared beam
- Each monitor holds 32 glass tubes
- One fly is placed into each tube



Micro Hybridization Incubator:
- Three experimental conditions:
  - 25.0 ºC
    - Stabilization and Recovery: Recovery from vial to tube transfer
  - 36.0 ºC
    - Preconditioning: Hsp upregulation
  - 38.5 ºC
    - Failure: No movement is recorded

Materials and Methods

Aim 1:
- Five genotypes of flies: WT31 ($MsrA^{+}B^{+}$), WT60 ($MsrA^{+}B^{+}$), A90 ($MsrA^{LOF}B^{+}$), B54 ($MsrA^{+}B^{LOF}$), AB46 ($MsrA^{LOF}B^{LOF}$)
- Flies placed into DAM system at 25.0 ºC for 15 minutes
- Flies stressed at 38.5 ºC for 30 minutes

Aim 2:
- Same genotypes used in Aim 1
- Flies placed into DAM system at 25.0 ºC for 15 minutes
- Flies preconditioned for 60 minutes at 36.0 ºC
- Flies recover at 25.0 ºC for 60 minutes
- Flies stressed at 38.5 ºC for 30 minutes

Failure Rates Without Preconditioning

5-9 days old:

Flies fail sooner in the absence of MsrB

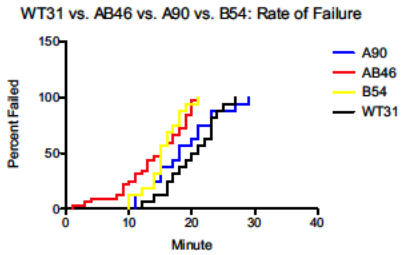


Figure 1: 32 AB46, 16 WT31, 16 A90, and 16 B54 flies were stressed for 35 minutes at 38 5 ºC AB46 was found to have a much slower failure rate than WT31 followed by B54 and A90 Therefore, MsrA and MsrB affect the failure rate of an organism n 80 A Log rank test was performed and the resulting p values were p 0 7076 for WT31 vs A90, p 0 0005 for WT31 vs B54, p 0 0002 for WT31 vs AB46, p 0 0069 for A90 vs AB46, p 0 0242 for A90 vs B54, and p 0 9400 for B54 vs AB46

35-39 days old:

In the absence of *any known* Msr activity, flies fail at a faster rate than wild-type

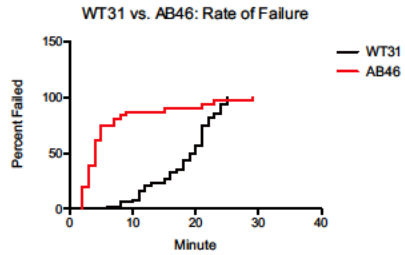


Figure 2: 31 AB46 and 48 WT31 flies aged between 35 and 39 days were stressed for 35 minutes at 38 5 ºC AB46 was found to have a much slower failure rate than WT31 This supports the hypothesis that MsrA and MsrB are involved in an organism's response to thermal stress n 79 A Log rank test was performed and the resulting p < 0 0001

Failure Rates With Preconditioning

5-9 days old:

Preconditioning is not affected by the presence of MsrA or MsrB and shortens failure rate

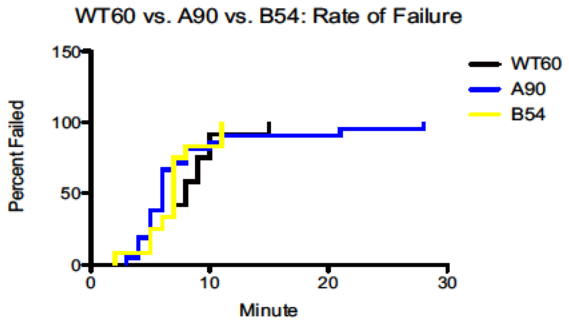


Figure 3 12 WT31, 21 A90, and 12 B54 flies were pre conditioned for 60 minutes at 36 0 ºC Following pre conditioning, flies were stressed for 30 minutes at 38 5 ºC All three genotypes were found to have a much slower failure rate with preconditioning than without n 45 A Log rank test was performed and the resulting p values were, p 0 6205 for WT60 vs A90, p 0 4913 for WT60 vs B54, and p 0 7572 for A90 vs B54

Discussion

- 5-9 days old flies lacking MsrB fail sooner than wild-type under thermal stress. However, flies lacking MsrA fail at a rate indistinguishable from wild-type, suggesting that MsrB – but not MsrA – plays a role in the animal's response to thermal stress (Figure 1).
- 35-39 day old flies lacking any known Msr activity fail at a significantly faster rate than wild-type (Figure 2). Additionally, the failure appears to be significantly more rapid in the older animals, suggesting that as an organism ages their thermal stress tolerance lowers (Compare Figures 1 and 2).
- 5-9 day old flies preconditioned to thermal stress unexpectedly failed faster than flies which had not been preconditioned (Figure 1 and 3). Also, there was no significant difference in failure rate for either MsrA or MsrB mutants). This finding suggests the presence of a mutation within our genotypes which alters the flies response to thermal stress following preconditioning or defective machinery (Figure 3).

Future Directions

- Repeat preconditioning experiments with standard wild-type genotypes
- Repeat failure experiments with 35-39 days old MsrA loss of function (LOF) and MsrB LOF flies
- Perform rescue experiments with all drivers and all genotypes: wild-type, MsrA LOF, MsrB LOF flies, and MsrA and MsrB LOF flies
- Examine whether the expression of MsrA and MsrB is tissue-specific using transgenic MsrA and MsrB loss of function flies

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