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DJ-1 AND SULFIREDOXIN IN OXIDATIVE STRESS MANAGEMENT IN *DROSOPHILA MELANOGASTER*

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Abstract

This study explores how mutations in the DJ-1 gene, linked to Parkinson's disease (PD), interact with oxidative stress and antioxidant mechanisms. We examined whether DJ-1 is a substrate for sulfiredoxin (Srx), an antioxidant that repairs oxidative damage. By testing mutant flies lacking DJ-1β, jafrac1 (Prdx-2), or Srx under oxidative stress, we found that the absence of Srx and DJ-1β led to varied stress responses, suggesting that other pathways may help maintain antioxidant defenses. These findings underline the complexity of DJ-1, Srx, and oxidative stress interactions, offering new directions for PD treatments to reduce oxidative damage.

Keywords: *Parkinson's disease; Dj-1, oxidative stress; Drosophila melanogaster*

1. Introduction

Parkinson's disease belongs to the neurodegenerative disease group and is widely prevalent around the world. According to Parkinson's Foundation statistics, 10 million people have Parkinson's disease. Parkinson's disease has a range of adverse symptoms, including motor, non-motor, behavioural, and cognitive dysfunctions. The disease is characterized by the loss of dopaminergic neurons in the substantia nigra of the midbrain and the accumulation of α- synuclein. α-synuclein is a protein located in the brain and can control synaptic functions.

Aggregation of α-synuclein in the brain damages the neurons and results in neuronal death [1]. Mutations in DJ-1 are associated with an autosomal recessive, early-onset form of familial Parkinson's disease. It is found that oxidized DJ-1 can stop the α-synuclein aggregation and prevent Parkinson's risk through its chaperone activity [2]. If there is a high amount of stress, the DJ-1 can be hyper-oxidised and cannot inhibit aggregation, and this might be one of the ways that neurodegenerative diseases are caused [3]. However, whether oxidation is reversible or not is unknown.

Another critical group of components fighting against oxidative stress is the peroxiredoxins, which belong to the peroxidase family of proteins. Peroxiredoxins are particularly interesting because they undergo a similar oxidation process to DJ-1, both oxidizing at cysteine residues. Under normal stress levels, their activated cysteine is oxidized to cysteine sulfenic acid. However, this sulfenic acid is further oxidised under high-stress conditions to sulfinic acid, rendering the peroxiredoxins inactive [4]. Sulfiredoxin (Srx), which recognizes the inactive peroxiredoxins, bind to the oxidized Cysteine residue and uses ATP as a cofactor to restore its activity. There are more than fifty-five substrates for sulfiredoxin in human organisms, but only peroxiredoxins (PRDX1-4) have been proven to be substrates for sulfiredoxin [5].

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Hypothesis: This leads us to consider that DJ-1 may also be a substrate for sulfiredoxin. If sulfiredoxin can help restore the activity of DJ-1, this interaction could represent a novel mechanism for enhancing cellular resistance to oxidative stress [4]. To investigate this, we examined the survival responses of different genotypes to various oxidative stressors, including paraquat, diethyl maleate (DEM), and hydrogen peroxide. In addition to assessing survival levels, we aimed to investigate the behavioral consequences of sulfiredoxin loss.

2. Materials and methods

Fly stocks and husbandry: All *Drosophila melanogaster* stocks were cultured on standard cornmeal and yeast agar at a controlled temperature of 25°C, 12-hour light/dark regime. The w^{1118} (Bloomington stock number #5905, #3605) strain was utilized as the control group. DJ-1β mutant flies (Bloomington stock number #33601) and sulfiredoxin mutant flies (Bloomington stock number #33537) were procured from the Bloomington Drosophila Stock Centre, Indiana [18]. The laboratory generated double mutants - $Srx^{\Delta 3}$/DJ-$1\beta^{\Delta 93}$ and sulfiredoxin mutants through P-element mutagenesis [19]. Jafrac1 (stock number #33537) flies were also created in the lab using P-element insertion. The *gstD1GFP* (Glutathione S-transferase D1 Green Fluorescent Protein) flies were generated using the method outlined in 2008 [20]. The GFP tag attached to GstD1 is a reporter transgene, enabling the visualization and quantification of GstD1 expression levels [21].

3. Results and discussion

Diethyl maleate significantly reduces longevity in $Srx^{\Delta 1\text{-}2}$ flies: Initially, we evaluated the responses of various genotypes to diethyl maleate (DEM) stress. We subjected 1–3-day-old male flies to a 10 mM DEM diet for 138 hours, recording survival counts twice daily (Fig. 1). Our observations demonstrated that $Srx^{\Delta 3}$ flies exhibited the highest resistance to DEM. In contrast, $Srx^{\Delta 1\text{-}2}$, Wild type, and DJ-$1\beta^{\Delta 93}$ flies displayed identical responses within the first 24 hours. Over time, $Srx^{\Delta 1\text{-}2}$ flies appeared most sensitive to DEM among the tested genotypes (p-value <0.05). DJ-$1\beta^{\Delta 93}$ flies demonstrated resistance, falling between $Srx^{\Delta 1\text{-}2}$ and $Srx^{\Delta 3}$ (p = 0.38431).

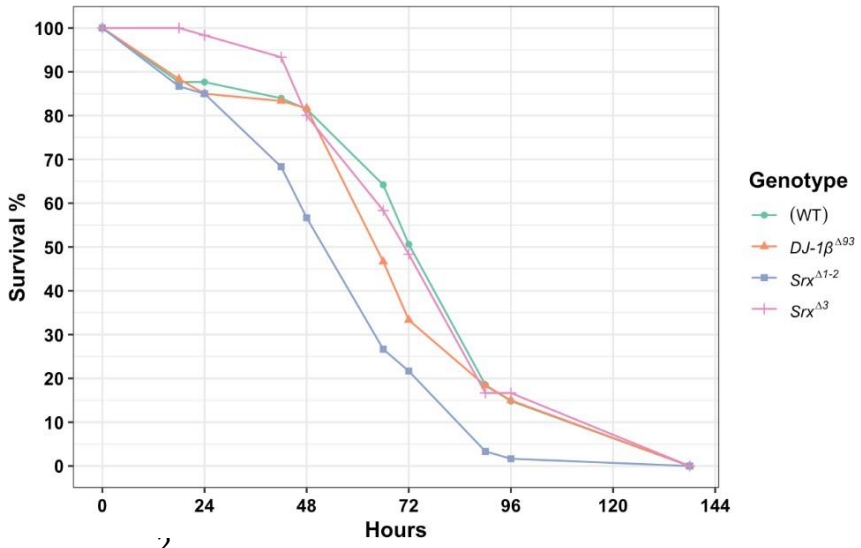

Fig. 1. Survival curves for different genotypes after the treatment with DEM

Jafrac1 flies exhibit extreme sensitivity to paraquat: In the following expe- riments, we included double mutants $Srx^{\Delta 3}$/DJ-$1B^{\Delta 93}$ and jafrac1 flies - which are homologues to human peroxiredoxin 2, to evaluate their resistance to 20 mM paraquat exposure over 168. All test subjects were 5-day-old male flies. Notably, the jafrac1 group, containing only 19 flies, exhibited the highest sensitivity to paraquat, with all individuals succumbing after 36 hours. In contrast, the double mutant flies initially showed substantial resistance, but their numbers sharply lowered post-24 hours, with complete mortality occurring by 90 hours of paraquat exposure. Both $Srx^{\Delta 1\text{-}2}$ and DJ-$1\beta^{\Delta 93}$ flies displayed similar survival patterns (p<0.05) (Fig. 2). While $Srx^{\Delta 3}$ and wild-type flies demonstrated notable resistance, the wild-type flies showed the highest endurance against paraquat, remaining alive after the 168-hour observation

period.

Hydrogen peroxide equally affects Srx$^{\Delta 3}$ and double mutant flies: In the latest test, male flies that were 5 days old were exposed to Hydrogen peroxide for 138 hours. Srx$^{\Delta 3}$ (p = 9.0e-07), DJ-1β$^{\Delta 93}$ (p = 0.00021), and Srx$^{\Delta 3}$/DJ-1β$^{\Delta 93}$ (p=1.2e-07) showed a decrease after 48 hours and were nearly deceased to zero by 96 hours ((Fig. 3). Contrary to previous results, Srx$^{\Delta 1\text{-}2}$ flies lived nearly as long as wild-type flies (p>0.05).

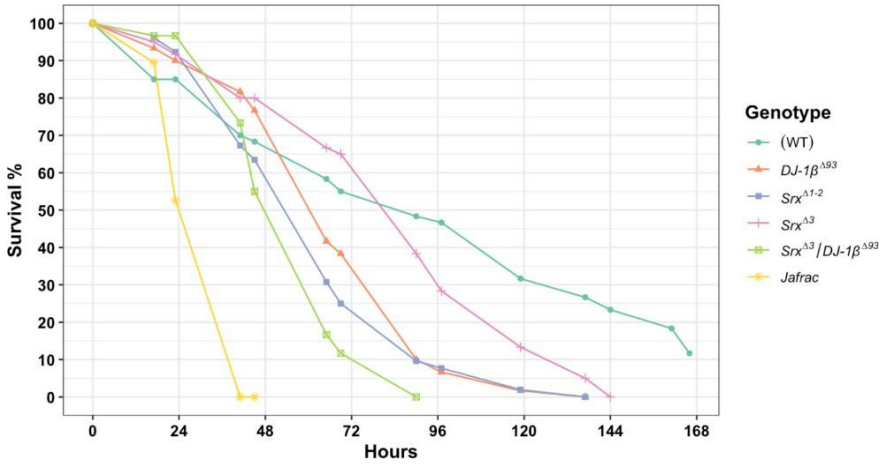

Fig. 2. Survival curves for different genotypes after the treatment of paraquat

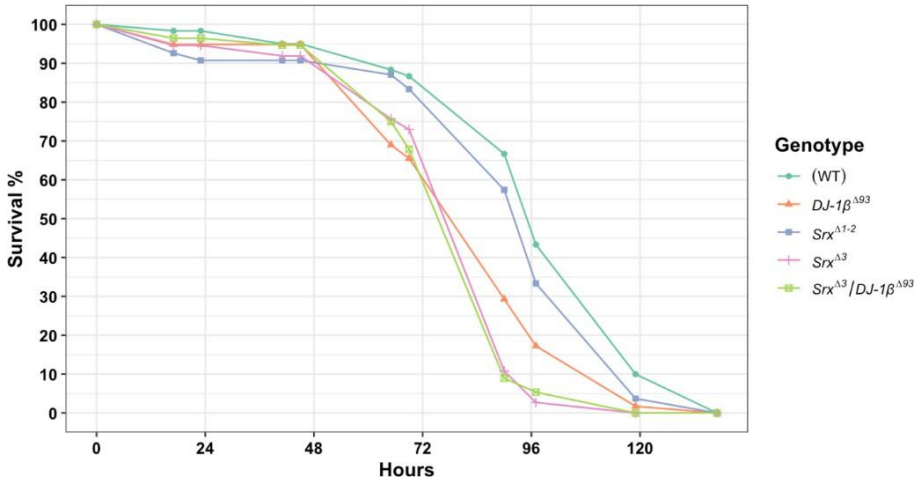

Fig. 3. Survival curves for different genotypes in 2% hydrogen peroxide over time

Fluorescence assay reveals genotype-specific oxidative stress responses: Upon observing the diverse results of different genotypes subjected to various treatments, we conducted fluorescence and BCA assays to assess whether these flies displayed elevated levels of oxidative stress and to ascertain if it was linked to their specific conditions. These assays were specifically carried out on flies crossed with the *gstd1GFP* flies. The fluorescence assay results, normalized to the Bradford assay results, are presented in Fig. 4. This analysis was performed to quantify the fluorescence intensity per milligram of protein across different genotypes (WT/GFP, Srx$^{\Delta 1\text{-}2}$/GFP, Srx$^{\Delta 3}$/GFP, jafrac1/GFP) under two conditions: DEM (diethyl maleate) and sucrose. Under the DEM condition, jafrac1/GFP showed the highest fluorescence intensity (mean ± SEM = 750±100 AU/mg), indicating a substantial increase in fluorescence compared to other genotypes. Srx$^{\Delta 1\text{-}2}$/GFP and Srx$^{\Delta 3}$/GFP exhibited moderate fluorescence intensities, whereas WT/GFP had the lowest fluorescence intensity in the DEM condition. In the sucrose condition, the fluorescence intensities were generally lower across all genotypes compared to the DEM condition. However, jafrac1/GFP still demonstrated a relatively high fluorescence intensity (mean±SEM = 500 ± 150 AU/mg) compared to the other genotypes. These results suggest that the jafrac1/GFP genotype exhibits significantly higher fluorescence, particularly under oxidative stress conditions induced by DEM, indicating a potential genotype-specific response to oxidative stress. The two-way ANOVA test showed significant effects of treatment (p = 7.51e-07) and genotype (p=0.000652) on fluorescence intensity but no significant interaction between treatment and genotype (p = 0.232028).

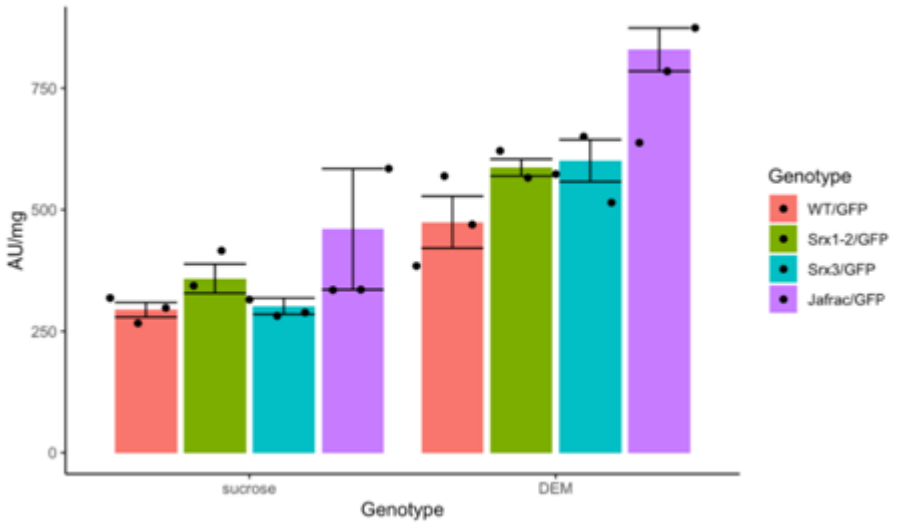

Fig. 4. Fluorescence - protein-based assay for different genotypes under DEM and sucrose

The role of DJ-1, sulfiredoxin and jafrac1 in paraquat-induced oxidative stress: Paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridinium dichloride) degrades in a NADPH-dependent manner, producing a persistent PQ radical that combines with oxygen to generate tre reactive oxygen species (ROS) superoxide anion [6]. We found that double mutants $Srx^{\Delta 3}$/DJ-1$\beta^{\Delta 93}$ flies were more sensitive to oxidative stress than their single mutant counterparts, indicating a complex interaction between these genes in managing oxidative stress and suggesting that the loss of both DJ-1 and sulfiredoxin increases vulnerability to oxidative damage (Fig. 2). The resistance of $Srx^{\Delta 3}$ flies to paraquat raises the possibility that DJ-1 (with the help of jafrac1, maybe) could stabilise or protect these flies, helping them tolerate oxidative stress. Despite this, the double mutants' better survival rate than jafrac1 mutants suggests that jafrac1 may partially compensate for the loss of DJ-1. When examining DJ-1 mutant flies, they also displayed sensitivity to paraquat, though to a lesser extent, further supporting the idea that jafrac1 may provide some compensatory protection. Two research studies were conducted on the sensitivity of DJ-1$\beta^{\Delta 93}$ flies to paraquat, yielding contrasting results [7, 8]. Our experiment confirmed that DJ-1β flies are sensitive to paraquat.

In our study, $Srx^{\Delta 1\text{-}2}$ flies were sensitive to paraquat, while $Srx^{\Delta 3}$ flies were resistant, demonstrating that specific mutations in the sulfiredoxin gene influence the flies' resistance or sensitivity to oxidative stress. $Srx^{\Delta 1\text{-}2}$'s sensitivity indicates that the response to paraquat can depend on $Srx^{\Delta 1\text{-}2}$ and might disrupted in its absence. However, we have not tested $Srx^{\Delta 1\text{-}2}$/DJ-1$\beta^{\Delta 93}$, so the effects of paraquat on those double mutant flies remain unknown. It can be done in future to see their interaction.

Additionally, jafrac flies, homologous to human peroxiredoxin-2 flies, which are the only confirmed substrates of sulfiredoxin, were the most sensitive to stress, exhibiting high oxidative stress compared to other flies before and after exposure to DEM (Fig. 4), [9, 4]. Our findings did not indicate a direct interaction between DJ-1 and sulfiredoxin, but they suggested that they might interact through alternative or parallel pathways.

Jafrac can act as a protective reserve against oxidative stress within cells, even without sulfiredoxin. If no sulfiredoxin exists, jafrac might be stored and used without reactivation. This result has been estimated because sulfiredoxin mutants displayed resistance while jafrac was sensitive. The presence of sulfiredoxin alone is insufficient to activate jafrac because the substrate is absent. As a result, these flies become more vulnerable to PQ. This vulnerability could explain the higher levels of oxidative stress observed in jafrac flies compared to other genotypes (Fig. 4). It is also worth mentioning that the jafrac group had only 19 flies, whereas other genotypes had 60, which may influence the interpretation of the data. These findings highlight the intricate balance between these pathways in protecting cells from oxidative damage and underscore the importance of proteins like DJ-1$\beta^{\Delta 93}$ and jafrac in managing oxidative stress. This was further supported by studies on peroxiredoxin-deficient C. elegans, where a decrease in lifespan was observed following paraquat exposure [10].

Performing co-immunoprecipitation and qPCR assays will be crucial to exploring the interactions between DJ-1, jafrac, and sulfiredoxin and shedding light on their potential role in oxidative stress resistance. Our results can suggest a compensatory mechanism between DJ-1 and jafrac.

Investigating sulfiredoxin and DJ-1β responses to diethyl maleate: Glutathione (L-3,-glutamyl-cysteinyl-glycine; GSH) is involved in cellular metabolism, essential in protecting cells from oxidative stress, and helps remove xenobiotics from the organism [11]. Diethyl maleate (DEM) is a chemical that decreases the effect of GSH by interacting with it [12]. Given DEM's known detrimental effects on lifespan [13], we examined how different fly genotypes respond to DEM exposure. However, research suggested that certain organisms, such as *C.elegans*, exhibit an adaptive response to DEM. This indicates that a small amount of DEM can extend the lifespan of *C.elegans*. Conversely, a high amount of DEM renders the organisms sensitive and decreases their survival rate [14]. Our observations revealed that $Srx^{\Delta 1\text{-}2}$ flies exhibited high sensitivity to DEM, correlating with their elevated oxidative stress levels compared to the resistant $Srx^{\Delta 3}$ flies (Fig. 1, 4). We demonstrated that DEM can significantly increase oxidative stress in $Srx^{\Delta 1\text{-}2}$ and $Srx^{\Delta 3}$ flies. It showed us that even if the stress level is high, flies with different genotypes can respond differently to DEM**.** Moreover, it was also found that DJ-$1\beta^{\Delta 93}$ flies were resistant to DEM. However, it is found that DJ-1 can affect the expression of glutathione reductase, which is the enzyme that helps to maintain the role of GSH. During the DJ-1 deficiency, GSH may be stored or activated by an alternative pathway so the DJ- 1 flies can resist DEM [15]. Unfortunately, the oxidative stress levels of DJ-$1\beta^{\Delta 93}$ flies have not been tested. In further experiments, it would be better to cross the DJ-$1\beta^{\Delta 93}$ flies with *gstD1GFP* flies and measure their ROS.

Exploring the impact of hydrogen peroxide on DJ-1β and sulfiredoxin mutant flies: H_2O_2 can impact the cell and raise cellular ROS levels by increasing oxygen radicals. One experiment found that flies with DJ-1β deficiency showed longer lifespans. They suggest that it's because of the alternative pathways which protect the organism even without the DJ-1β [16]. However, we found different results in our experiment (Fig. 3). Initially, all genotypes exhibited resistance for the first 48 hours, followed by a decline. This indicates that their defense systems were likely active during the initial 48-hour period but eventually became inactive. This can be an adaptive response to H_2O_2. In this trial, $Srx^{\Delta 3}$, DJ-$1\beta^{\Delta 93}$, and $Srx^{\Delta 3}$/DJ-$1\beta^{\Delta 93}$ displayed sensitivity to hydrogen peroxide.

Interestingly, in the other experiments with DEM and PQ, $Srx^{\Delta 3}$ showed resistance; however, against the H_2O_2, they could not survive and died quickly. However, this experiment can support our hypothesis that DJ-1 is a substrate for Srx because they have shown similar results in this assay. It also might suggest that another pathway or mechanism interacted with $Srx^{\Delta 3}$ and showed sensitivity against H_2O_2. Notably, while it was known that DJ-$1\beta^{\Delta 93}$ flies are sensitive to hydrogen peroxide, there was no research regarding the sensitivity of sulfiredoxin flies [17, 7]. Future research can identify the other double mutants' reactions against ROS.

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