Redox Biology 17 (2018) 377–385

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research paper

Neuron-specific regulation of superoxide dismutase amid pathogen-induced gut dysbiosis

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ABSTRACT

Superoxide dismutase, an enzyme that converts superoxide into less-toxic hydrogen peroxide and oxygen, has been shown to mediate behavioral response to pathogens. However, it remains largely unknown how superoxide dismutase is regulated in the nervous system amid pathogen-induced gut dysbiosis. Although there are five superoxide dismutases in *C. elegans*, our genetic analyses suggest that SOD-1 is the primary superoxide dismutase to mediate the pathogen avoidance response. When *C. elegans* are fed a *P. aeruginosa* diet, the lack of SOD-1 contributes to enhanced lethality. We found that guanylyl cyclases GCY-5 and GCY-22 and neuropeptide receptor NPR-1 act antagonistically to regulate SOD-1 expression in the gustatory neuron ASER. After *C. elegans* ingests a diet that contributes to high levels of oxidative stress, the temporal regulation of SOD-1 and the SOD-1–dependent response in the gustatory system demonstrates a sophisticated mechanism to fine-tune behavioral plasticity. Our results may provide the initial glimpse of a strategy by which a multicellular organism copes with oxidative stress amid gut dysbiosis.

1. Introduction

Gut dysbiosis is caused by an imbalance of beneficial and harmful microbes in the intestine [1,2]. In addition to digestive tract–related morbidity [2–6], recent studies have revealed that dysbiosis of gut microbiota plays a role in emotional and cognitive behaviors [7–10] and is found in patients with neurodegenerative diseases [11–13].

In the laboratory, *C. elegans* is often reared on a petri dish that contains a lawn of non-pathogenic *Escherichia coli* OP50 [14]. Recent studies reveal that pathogenic bacteria *Pseudomonas aeruginosa*, which are frequently found in the habitats of nematodes [15,16], can colonize the intestine of *C. elegans* [17–20]. Switching *C. elegans* from a diet of *E. coli* to a diet of *P. aeruginosa* creates a condition that mimics gut dysbiosis [21–23]. The change in the microbial composition in the intestine activates host responses [22,24–26]. For example, intestinal accumulation of *P. aeruginosa* activates the production of reactive oxygen species (ROS) [22,27–29]. The ROS burst further elevates the expression of antioxidant enzymes, including superoxide dismutase SOD-1 [22]. SOD-1 is an enzyme that converts superoxide into less-toxic hydrogen peroxide and oxygen [30–32]. Indeed, animals carrying a *sod-1* deletion elicit a strong aversive response to *P. aeruginosa*, presumably due to their reduced capacity to ameliorate elevated ROS [22].

ASE neurons are a pair of chemosensory neurons defined by their ability to detect water-soluble cues and transmit this information to evoke a specific behavioral response [33]. Therefore, they are often regarded as the gustatory (taste) neurons in *C. elegans*. Although ASE neuron cell bodies are symmetrically positioned in the lateral ganglia region of *C. elegans* brain, the molecular compositions of left (ASEL) and right (ASER) gustatory neurons are not identical [34,35]. ASEL and ASER gustatory neurons express distinct members of a putative chemoreceptor gene family and respond in distinct manners to different cues [36,37]. For instance, animals devoid of the ASER neuron elicited a heightened aversive response to *P. aeruginosa* [22]. In contrast, animals lacking the ASEL neuron did not show the heightened pathogen avoidance response. SOD-1 is present in the nervous system, and SOD-1 expression is elevated in the ASER neuron by *P. aeruginosa*. It is possible that ASER-specific chemosensory receptors activate SOD-1, but experimental data have yet to support this notion.

Another poorly understood phenomenon concerns the following observation: After extended feeding on *P. aeruginosa*, ASER-specific SOD-1 elevation becomes diminished. Reduction of SOD-1 is coupled with the initiation of aversive behavior to *P. aeruginosa* [22]. However, the mechanism of SOD-1 down-regulation after extended *P. aeruginosa* feeding remains unknown. It has been shown that deletion in neuropeptide receptor NPR-1 results in extended feeding on *P. aeruginosa* [21,26]. Neuropeptide receptor NPR-1 is *C. elegans* neuropeptide Y receptor, which regulates food satiety and stress response [21,26,38,39]. The NPR-1 receptor is present in the ASER neuron [40]. We hypothesize

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https://doi.org/10.1016/j.redox.2018.05.007
Received 1 May 2018; Received in revised form 12 May 2018; Accepted 14 May 2018
Available online 19 May 2018

that NPR-1 may play a role in modulating the SOD-1–dependent behavioral response to *P. aeruginosa* by down-regulating SOD-1 expression in the ASER neuron.

There are three zinc–copper superoxide dismutase isoforms (SOD-1, SOD-4, and SOD-5) and two manganese superoxide dismutase isoforms (SOD-2 and SOD-3) in *C. elegans* [41]. Based on their subcellular localization, the zinc–copper isoforms are further classified as cytoplasmic (i.e., SOD-1 and SOD-5) and extracellular/secreted (i.e., SOD-4) superoxide dismutases. Previous studies suggest these isoforms are expressed rather ubiquitously, including in the nervous system. For example, SOD-1 appears to be expressed in most cells of the worm [42,43]. SOD-5 expression is inducible and has been detected in a small subset of neurons [42]. SOD-1 has been previously suggested to act in the gustatory neuron ASER to regulate *C. elegans* behavioral response to *P. aeruginosa* [22]. Do additional superoxide dismutases in *C. elegans* mediate the behavioral response to *P. aeruginosa*? And what are the molecular mechanisms that regulate the superoxide dismutase–dependent response to pathogen-induced gut dysbiosis?

In this study, we found that SOD-1 and SOD-5 are both present in the gustatory neuron ASER. Our genetic analyses suggest that SOD-1 plays a primary role in regulating the behavioral response to *P. aeruginosa*. SOD-1 is induced in the nervous system and in the intestine by oxidative stress. Lack of SOD-1 contributes to enhanced lethality after *C. elegans* is fed the *P. aeruginosa* diet. ASER-specific guanylyl cyclases GCY-5 and GCY-22 activate SOD-1 expression, whereas neuropeptide receptor NPR-1 promotes the reduction of SOD-1 in the ASER neuron. Therefore, we have identified the regulatory mechanisms that contribute to the activation and reduction of superoxide dismutase. Our results also suggest that zinc–copper superoxide dismutase plays a major role in mediating the behavioral response amid pathogen-induced gut dysbiosis.

2. Results

2.1. Superoxide dismutase isoform SOD-1 plays a primary role in behavioral response to P. aeruginosa

We began our study by investigating the role of additional superoxide dismutases in *C. elegans*. Deletion in *sod-1* contributes to a heightened pathogen avoidance response [22]. Therefore, we first obtained deletion mutations of *sod-2*, *sod-3*, *sod-4*, and *sod-5* (Fig. 1A). We then exposed the mutant animals to a lawn of *P. aeruginosa* on a petri dish and compared their *P. aeruginosa* lawn avoidance phenotypes (Fig. 1B). We found that *sod-2* (*gk257*) and *sod-4* (*gk101*) elicited phenotypes similar to wild type. In contrast, we found that *sod-3* (*tm760*) and *sod-5* (*tm1146*) showed enhanced pathogen avoidance responses at 7 h. However, the *P. aeruginosa* avoidance responses of *sod-3* (*tm760*) and *sod-5* (*tm1146*) were not as strong as that of *sod-1* (*tm776*) (Fig. 1B). Since both zinc–copper superoxide dismutase SOD-1 and SOD-5 play roles in regulating *C. elegans* avoidance response to *P. aeruginosa*, we generated a *sod-1* and *sod-5* double mutant and fed the double mutant *P. aeruginosa*. We found that *sod-1* (*tm776*); *sod-5* (*tm1146*) elicited a pathogen avoidance response similar to that elicited by *sod-1* (*tm776*) alone (Fig. 1C). We then introduced a 2.9 kb *sod-1* genomic fragment that encompasses the coding and the 5′ and 3′ regulatory regions of *sod-1* [22] into the *sod-1* (*tm776*); *sod-5* (*tm1146*) double mutant. We were able to rescue the pathogen avoidance response of the *sod-1* (*tm776*); *sod-5* (*tm1146*) double mutant to the wild type level (Fig. 1C). Because a genomic fragment of *sod-1* rescues the pathogen avoidance response of *sod-1*; *sod-5*, SOD-5 may act as an auxiliary isoform to regulate *C. elegans* pathogen response.

Finally, we compared the pathogen avoidance phenotype of a *sod-1*; *sod-2*; *sod-3*; *sod-4*; *sod-5* quintuple mutant to that of the *sod-1*; *sod-5* double mutant and to the *sod-1* single mutant. We found there are no statistically significant differences between the quintuple mutant, the *sod-1*; *sod-5* double mutant, and the *sod-1* single mutant with respect to avoidance responses to *P. aeruginosa* (Supplementary Fig. 1). Since the quintuple mutant does not further enhance *sod-1* single mutant phenotype, this suggests that SOD-1 is the primary superoxide dismutase to regulate the behavioral avoidance response to *P. aeruginosa*.

2.2. SOD-1 and SOD-5 are present in overlapping amphid sensory neurons

Our data indicate that SOD-5 may act as a redundant zinc–copper superoxide dismutase in the context of pathogen avoidance. If this is the case, SOD-5 is likely present in a similar set of neuronal cells to SOD-1. We therefore sought to investigate the expression pattern of SOD-5. We first generated an integrated reporter strain that contains an RFP reporter driven by the promoter of *sod-5* (*sod-5p*::RFP). We examined the fluorescence signals of *sod-5p*::RFP and detected no obvious *sod-5p*::RFP fluorescence signals when the animals were reared under normal growth condition. However, when the animals were starved, we found *sod-5p*::RFP fluorescence signals in the pharynx and in the nervous system (Supplementary Fig. 2). The fluorescence signals of *sod-5p*::RFP are present in the neuron ganglion posterior to the anterior bulb of the pharynx. Several of the neurons have projections extending anteriorly to the tip of the nose, a morphology that resembles amphid sensory neurons. To determine if SOD-5 is present in the amphid sensory neurons, we performed dye-filing experiments using starved *sod-5p*::RFP animals. We found that *sod-5p*::RFP fluorescence is present in the ASJ neurons. We also observed a weak signal of *sod-5p*::RFP in the ASE neuron pair (Fig. 2A). To further compare the expression pattern of SOD-5 to that of SOD-1, we performed dye-filling experiments using the *sod-1p*::*sod-1* cDNA::RFP reporter strain. Similar to prior results [22], we found that SOD-1 is present in the ASER neuron (Fig. 2B). These results suggest that SOD-1 and SOD-5 are both present in ASER gustatory neurons.

Previous investigation suggests that SOD-5 expression is inducible [42]. Consistent with the prior result, our data show that *sod-5p*::RFP is at undetectable levels under the normal growth condition. We found that the SOD-5 reporter is induced in the nervous system amid starvation and that SOD-5 is present at low levels in the ASE neuron pair. Previous work has also shown that SOD-1 contributes almost 80% of the total *sod* mRNA expression and 80% of the total SOD activities [42]. Together with our results, we reason that SOD-5 likely plays an auxiliary role in mediating the zinc–copper superoxide dismutase–dependent response to *P. aeruginosa*.

2.3. SOD-1 is induced by reactive oxygen species in the nervous system and intestine

SOD-1 is an enzyme that converts superoxide into less-toxic hydrogen peroxide and oxygen. We hypothesized that SOD-1 expression is activated by an increase in ROS. To test this, we exposed the SOD-1 fluorescence reporter strain (*sod-1p*::*sod-1* cDNA::RFP) to 100 μM paraquat and measured the fluorescence intensity of SOD-1::RFP. We found that SOD-1 is elevated by paraquat in the nervous system and in the intestine (Fig. 3). We also tested whether exposing *C. elegans* to *P. aeruginosa* induces SOD-1 expression. Similar to previous report [22], we found that the fluorescence signals of SOD-1::GFP are elevated in the ASER neuron after 2 h *P. aeruginosa* exposure. In contrast, intestinal SOD-1::GFP is elevated after 8 h (Supplementary Fig. 3A and B). These results suggest that SOD-1 may act in the nervous system and/or in the intestine in response to an increase in pathogen-induced oxidative stress.

2.4. SOD-1 alleviates lethality triggered by pathogen-induced oxidative stress

Exposure to *P. aeruginosa* elevates oxidative stress [22,29] and contributes to early demise of *C. elegans* [21,44,45]. To determine the tissue-specific requirements of SOD-1 in alleviating pathogen-induced