PART II

OXIDATIVE STRESS IN INVERTEBRATES

18

ENDOCRINE CONTROL OF OXIDATIVE STRESS IN INSECTS

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18.1 INTRODUCTION

The implications for the toxic properties of oxygen were unknown until Gershman's seminal proposal of the free radical theory of oxygen toxicity in 1954, which suggested that the poisonous nature of oxygen is due to the partially reduced forms of oxygen [1]. In the same year Commoner et al. [2] reported weak but detectable electron paramagnetic resonance (EPR) signal in lyophilized biological materials, and this was attributed to the presence of free radicals. These discoveries triggered intense research into the role of free radicals in biological systems. Since then, a large body of evidence has been accumulated that living systems have not only adapted to a coexistence with free radicals but have also developed various mechanisms for advantageous use of free radicals in various physiological functions. Oxygen free radicals generally termed as reactive oxygen species (ROS) as well as reactive nitrogen species are products of normal cellular metabolism. The harmful effect of ROS causing potential biological damage is called oxidative stress. This occurs in biological systems when there is an overproduction of ROS on one side and a deficiency of enzymatic and nonenzymatic antioxidants on the other. In other words, oxidative stress results from metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant and antioxidant reactions in living organisms.

18.2 ENDOGENOUS SOURCES OF OXIDATIVE STRESS

Oxidative stress in insects is an unavoidable by-product of the aerobic lifestyle, because the superoxide anion ($O_2^{\bullet -}$) and H_2O_2 are formed whenever molecular oxygen chemically oxidizes electron carriers. The superoxide anion ($O_2^{\bullet -}$) radical is generated by the one-electron reduction of O_2. Cellular sources of ROS production include plasma membrane NADPH oxidase and intracellular cytosolic xanthine oxidase, peroxisomal oxidases, endoplasmic reticular oxidases, and mitochondrial electron transport components. ($O_2^{\bullet -}$) production can also be due to autoxidization of catecholamines, ubihydroquinone, hemoproteins, and flavin enzymes [3]. Other endogenous sources of ROS include stress and starvation. In all these cases the generation of free radicals is implied. A free radical is unstable and highly reactive and contains one or more unpaired electrons. It can be formed by any of the processes outlined below:

a. Homolysis of covalent bonds

$$A : B \rightarrow A^{\bullet} + B^{\bullet}$$

b. Addition of a single electron to a neutral atom

$$A + e \rightarrow A^{-\bullet}$$

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c. Loss of a single electron from a neutral atom

$$A \rightarrow A^{+\bullet} + e$$

In insects the following free radicals are generated as a result of the above processes:

Superoxide anion generation:

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

$$H_2O_2 \rightarrow HO_2^{\bullet} + e^- + H^+$$

$$O_2 + NADPH \rightarrow O_2^{\bullet -} + NADP + H^+$$

Hydroxyl radical generation:

$$O_2^{\bullet -} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2 [\text{Haber–Weiss reaction}]$$

$$H_2O_2 + Fe^{2+}/Cu^+ \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}/Cu^{2+} [\text{Fenton reaction}]$$

Hydrogen peroxide generation:

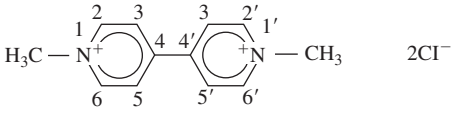
$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

18.3 EXOGENOUS SOURCES OF OXIDATIVE STRESS

Environmental pollutants that include a variety of biochemical synthetic compounds used in industry and agriculture can affect nontarget organisms. These include metals, metalloids, and numerous other organic compounds. In general, halogenated alkanes and alkenes that are effluents from industrial sources and agriculture have been shown to induce oxidative stress by generating ROS in nontarget species. A comprehensive review of oxidative stress from environmental pollutants has been provided by Ahmad [4]. Here, we shall focus on oxidative stress in herbivorous insects that can be caused both by prooxidant plant allelochemicals and by various herbicides and/or insecticides. Plant phenolic compounds, particularly flavanoids and tannins, have long been associated with plant defense against herbivores [5]. Toxic phenoxyl radicals are formed via oxidative processes owing to their ability to initiate free radical chain reactions in the membrane and the propensity to cross-link with a variety of molecules [6, 7]. The midgut of insect herbivores is a highly oxidizing environment. Hence diet supplementation of lepidopteran larvae *Helicoverpa zea* and *Spodoptera littoralis* with phenolic acids was found to increase various indicators of oxidative stress in gut tissues [8, 9]. The toxicity of phenolics results from several different modes of action including binding and oxygen radical formation. In general, phenolics can participate in four major types of bonds: hydrophobic, hydrogen, ionic, and covalent [10]. The formation of oxygen radicals is another important mode of phenolic action. Generally, almost any oxidation of phenolics can result in the generation of superoxide anion radicals because the reactive semiquinone can donate an electron to molecular oxygen. The superoxide anion so generated can further lead to the generation of additional radical species, including hydroxyl radicals, as described above. Thus, the propensity of phenolics to generate radicals depends on whether they are ionized or oxidized. The oxidation and ionization of phenolics depends on their phenolic structure, the physicochemical conditions under which the reactions take place, including hydrogen ion availability (pH), electron availability (E_h, or redox potential), and the concentration of antioxidant enzymes as well as nonenzymatic oxidants and reductants. Additionally, furanocoumarins are also known to generate free radicals in herbivores because they are photoactive prooxidants. These are found in plants of Apiaceae and Rutaceae. Among herbicides, paraquat is well known to generate oxidative stress in insect species [4].



Paraquat dichloride (BP^{2+})

$$BP^{2+} + e^- \rightarrow BP^{+\bullet}$$

$$BP^{+\bullet} + O_2 \rightarrow BP^{2+} O_2^{\bullet -}$$

18.4 DEFENSES AGAINST OXIDATIVE STRESS

Defenses elaborated against oxidative stress are termed antioxidative defenses. By definition, then, an antioxidant can be defined as any substance that can either delay or prevent the oxidation of a substrate when it is present in small amounts relative to the amount of the substrate. According to Halliwell and Gutteridge [11], antioxidants can act at several different levels in the oxidative sequence, and they may have multiple mechanisms of action. Thus, antioxidative defenses could essentially be divided broadly into two main mechanisms: the enzymatic—which maintain harmless levels of activated oxygen species by reducing excess to H_2O—and the nonenzymatic—which are essentially free radical

scavengers designed to remove free radicals generated during reductive processes or during deleterious reactions between excess ROS and cellular macromolecules.

18.4.1 Enzymatic Antioxidative Mechanisms

Insects, like other animals, possess a suite of enzymes that are directed toward the removal of various radicals [12–17]. These include superoxide dismutase, catalase, ascorbate peroxidase, glutathione *S*-transferase peroxidase, etc.

Superoxide dismutase (SOD): Superoxide dismutases are mainly of two main types—the Cu/Zn SOD (located in the cytosol) and the MnSOD (localized in the mitochondria). These enzymes catalyzes the following reaction:

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Catalase (CAT): Catalase is a 24-kDa homotetrameric enzyme with a heme-iron active center, whose main function is to decompose toxic hydrogen peroxide [18]. This enzyme is localized notably in organelles such as peroxisomes. Hydrogen peroxide is eliminated by catalase as follows:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Ascorbate peroxidase (APOX): This enzyme catalyzes the oxidation of ascorbic acid with the concurrent reduction of hydrogen peroxide and could serve an important function in removal of hydrogen peroxide in insects [19]. Regeneration of reduced ascorbate is achieved by the enzyme dehydroascorbate reductase. Ascorbate by itself is known to scavenge singlet oxygen, superoxide, and hypochlorite as well as water-soluble radicals such as peroxyl, hydroxyl, thiyl, suphenyl, and nitroxide radicals [13,20–25]. Thus, in addition to its peroxidase activity, the efficient recycling of ascorbate itself gives it a powerful antioxidant role.

Glutathione S-transferase peroxidase (GSTPx): Vertebrates contain a selenium-dependent glutathione peroxidase (GPOX) that removes both hydrogen peroxide and lipid peroxides [12]. Insects have a glutathione *S*-transferase with peroxidase activity (GST-px). This enzyme can metabolize lipid peroxides but is unreactive toward hydrogen peroxide [12, 15, 26, 27].

Thioredoxin/thioreductase system (Trx/TrxR): To maintain its preferred redox state, the insect cells activate the NADPH-dependent thioredoxin/thioreductase enzyme system, which rapidly restores the modified thiols to their unmodified reduced state [25, 28].

18.4.2 Nonenzymatic Antioxidative Mechanisms

In addition to the classical antioxidant enzyme systems, a number of small molecules also play a significant role in scavenging ROS and curbing the deleterious effects of oxidative stress. Specifically in insects some of these small molecules are plant derived, while others can be synthesized by insects:

Carotenoids: The carotenoids constitute a family of conjugated polyenes. Carotenoids could be vitamin A precursors and are able to quench singlet oxygen. They may also directly react with peroxyl radicals. In plants their role has been well documented as a free radical scavenger, but the specific role in insect systems remains a speculation.

α-Tocopherol: The natural α-tocopherol is the 2R,4′R,8′R-α-tocopherol. This lipid-soluble vitamin is very reactive in membranes and can react with lipid peroxyl radical $LOO^{\cdot}$ to form the relatively stable radical that probably does not react.

$$\alpha\text{-tocopherol} + LOO^{\bullet} \rightarrow LOO - \alpha\text{-tocopherol}$$

Ascorbic acid: Insects cannot synthesize ascorbic acid and depend on dietary sources for their supply of this essential antioxidant. In the presence of reactive species, ascorbic acid (or its conjugated base, ascorbate) is a one-electron donor and is oxidized in semidehydroascorbate radical, a molecule stabilized by the delocalization of the electrons between the three oxygen atoms. The semidehydroascorbate is converted back into ascorbic acid in the presence of glutathione and dehydroascorbate reductase:

$$\text{Asc} + \text{radical} \rightarrow \text{semidehydroascorbate} + \text{reduced radical}$$

$$\text{Asc} + \text{HOCL} \rightarrow \text{semidehydroascorbate} + H_2O + \text{HCl}$$

Glutathione (GSH): Glutathione is a very important free radical scavenger. This is a tripeptide γ-glutamyl-cysteine-glycine and is one of the most abundant low-molecular-weight thiols present in the cell. Reduced GSH is characterized by its reactive thiol group, and as an effective reductant it plays an important role in a variety of detoxification processes. GSH readily interacts with free radicals and oxidizing compounds such as H_2O_2, $O_2^{\bullet -}$, $OH^{\cdot}$, and carbon radicals including protection against lipid peroxide damage [14, 25, 29]. In the presence of the radicals the reduced form (GSH) is oxidized (GSSG) and can then be recycled back in a NADPH-dependent reaction catalyzed by glutathione reductase or by the thioredoxin reductase systems [30].

In addition to the antioxidant mechanisms and systems described above, insects also possess several water-soluble molecules (uric acid, carbohydrates, polyols) and iron binding proteins (ferritin and transferrin) that also serve crucial antioxidant functions [14].

18.5 REGULATION OF DEFENSES AGAINST OXIDATIVE STRESS

Oxidative stress triggers a range of physiological, pathological, and adaptive responses in insect cells either as a result of cellular damage or through specific signaling molecules. These responses ultimately modulate transcriptional outputs to influence and induce antioxidant systems. In the past couple of decades, a number of transcription factors and signaling pathways have been identified and delineated to mediate critical transcriptional responses to oxidative stress. These signaling pathways include mitogen-activated protein kinases (MAPKs) P13K/Akt, protein kinase C (PKC), protein53 (p53), nuclear factor κB (NF-κB), activator protein-1 (AP-1), and redox regulation by redox factor-1 (Ref-1) and the Nrf2-mediated antioxidant response. Nrf2 (nuclear factor erythroid 2 related factor 2) belongs to a group of specialized transcription factors termed xenobiotic-activated receptors (XARs). These recognize specific xenobiotics and coordinate the transcription of batteries of genes. The specifics of activation at the gene level of these signaling pathways are beyond the scope of this particular chapter. Here, we focus on the role that insect hormones play in the regulation of defenses against oxidative stress.

18.6 INSECT HORMONES AND THEIR ROLE IN THE CONTROL OF OXIDATIVE STRESS

The insect endocrine system produces various hormones and biologically active factors that can be divided into three main groups [31] including (a) ecdysteroids, steroid hormones, produced primarily by prothoracic glands and partially also by several other tissues. They control mainly molting, development, metamorphosis, and reproduction and are also involved in a number of diverse processes. (b) Juvenile hormones (JHs), a family of acyclic sesquiterpenoids, the principal products of the corpora allata—a retrocerebral gland—are involved in the regulation of metamorphosis and reproductive processes such as control of gonadal development and vitellogenin synthesis. Additionally, the roles of JH have expanded to include caste determination, behavior, diapause, and various polyphenisms [32]. And finally, insects also produce (c) neurohormones, a large group of peptidergic compounds produced by specialized secretory neurons, which are most abundant in the brain but do occur throughout the whole nervous system. They control a number of biochemical, physiological, and behavioral events in the insect body including those mentioned above for ecdysteroids and JHs [33]. The classification of neurohormones is not uniform, but they are ordinarily categorized according to their functions. One of the best-defined groups of neurohormones associated with stress responses are metabolic neuropeptides belonging to the AKH/RPCH family (adipokinetic hormone/red pigment concentrating hormone family). A major function of these small peptides (octa-, nona-, or decapeptides), which are synthesized and released from an endocrine retrocerebral gland corpora cardiaca, is the control of insect metabolism. However, they are pleiotropic, with a number of actions in addition to their metabolic role. Generally, they behave as typical stress hormones by stimulating catabolic reactions (mobilize lipids, carbohydrates, and/or certain amino acids), making energy more available, while inhibiting synthetic reactions. They mobilize entire energy reserves to combat the immediate stress problems and suppress processes that are momentarily less important and could, if allowed to continue, even draw on the mobilized energy. These biochemical stress reactions are accompanied by activation of physiological stress response that includes stimulation of heart beat [34], increase of muscle tonus [35], stimulation of general locomotion [36], enhancement of immune response [37, 38], and some others. Recently it has been found that AKHs are also involved in the control of oxidative stress (OS) in insects [see 39].

18.7 ROLE OF ADIPOKINETIC HORMONES IN INSECT OXIDATIVE STRESS

An active role of AKHs in protection of insects against OS was deduced from results of a set of papers published recently (see below). These results reveal that oxidative stressors increase the level of AKHs and, additionally, that exogenous AKHs mitigate OS biomarkers in insect body experimentally enhanced by application of the stressors. These facts indicate that there is a feedback regulation between an oxidative stressor and AKH actions, and that AKHs are involved in the activation of an antioxidant protection mechanism in insects.

Application of the oxidative stressors elevates the titer of AKHs in hemolymph. The effect is reported for paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride hydrate) [40, 41] a redox cycling herbicide that is commonly used to create conditions of OS in insect body, and also for *Galanthus nivalis* agglutinin and *Bacillus thuringiensis* toxin [40] and for endosulfan and malathion [42]—insecticides with an OS effect. The intensity of AKH level elevation varies depending on insect species, type of oxidative stressor, or time of the stressor incidence. For example, feeding of genetically modified potatoes containing the Cry 3Aa *Bacillus thuringiensis* toxin elevates the AKH level in hemolymph of the Colorado potato beetles *Leptinotarsa decemlineata* up

to 6-fold and treatment by paraquat about 2.7-fold within 4 hours after the treatment [40]. Similarly in the bug *Pyrrhocoris apterus* paraquat increases the AKH level in hemolymph about 5 times (again 4 hours after the treatment) [41] and insecticides endosulfan and malathion about 1.5 times 24 hours after the treatment [42].

Even larger variability was recorded when level of AKH was measured in the corpora cardiaca or the whole CNS (including the corpora cardiaca). In *L. decemlineata* feeding of both the Cry 3Aa toxin and *Galanthus nivalis* agglutinin elevates the level up to 10 times and application of paraquat just 1.9 times [40]. In *P. apterus* the same application has no effect on the AKH level in the CNS (4 hours after the treatment) [41], and treatment with the insecticides endosulfan and malathion elicits only a slight increase in the CNS [42]. On the other hand, the differences between the stress-evoked AKH levels in CNS and hemolymph are not so surprising, because the whole amount of AKH seems to be substantially distinct in those two body parts. It is reported there is about 200 times lower AKH amount in hemolymph than in CNS of *P. apterus* [43]. In addition, no positive correlation is shown for the same species between the AKH level in hemolymph and CNS [44]. This result is in good agreement with the suggestion that a coupling between release and biosynthesis of the AKHs in adipokinetic cells of the corpora cardiaca is very loose or does not even exist [45].

Questions arise on the possible mode of the feedback action on the biochemical pathways by which the enhanced AKH level mitigates the OS. While the mechanisms of AKH action leading to energy mobilization have been documented by a number of publications [46–48, etc.], almost nothing is known about the stress-evoked AKH actions that do not include rapid production and subsequent consumption of energy. Other than OS, stress-induced elevation of AKH titer was demonstrated in the locust *Schistocerca gregaria* and *P. apterus* challenged with an insecticide [42, 49–51], excessive KCl [49], and photophase interruption and exposure to constant darkness [52].

The suggestion that AKHs are involved in the activation of protective antioxidative mechanisms derived from the effect of oxidative stressors on AKH level in the insect body is supported by a series of experiments demonstrating direct involvement of AKHs in the modulation of OS biomarker levels. Only a few biomarkers have been studied in the relationship to AKH actions so far. One of the most convenient is glutathione. It is a low-molecular-weight thiol (see Section 18.4.2) found in the cytosol and other aqueous phases of various living systems [53, 54]. The level of GSH in insect hemolymph is significantly increased (about twice) after AKH injection but significantly depressed (about 2–3 times) after paraquat treatment both in *L. decemlineata* [40] and *P. apterus* [41] hemolymph. On the other hand, coinjection of AKH together with paraquat results in enhanced GSH content back to the control level. A very similar picture is obtained when the insecticides endosulfan and malathion are used [42]. It appears that AKH is able to directly or indirectly enhance efflux of reduced GSH into the insect hemolymph as demonstrated for the pancreatic vertebrate hormone glucagon that stimulates massive GSH efflux from the liver into the bloodstream [55]. However, it is quite possible that GSH alone (induced by AKH) could not be the one to confer enhanced antioxidant capacity to the hemolymph.

Another important marker of OS is protein carbonylation, whose level illustrates the oxidative damage of the system. Carbonyls are formed from amino groups in the side chain of certain amino acids that are exposed to ROS [56]. Application of paraquat into both *L. decemlineata* and *P. apterus* body, as well as application of Cry 3Aa and *Galanthus nivalis* toxins in the food of *L. decemlineata*, significantly enhance carbonyl contents in hemolymph, but coinjection of paraquat with AKH decreases their levels to those found in control groups [40, 41]. It is interesting that AKH injection alone does not change the carbonyl contents to those below control values. This indicates that possibly a stressor action is needed for AKH to potentiate the response, as in case of phenoloxidase activity [37], lipid store mobilization rate after injection and/or topical application of external AKH [57], and modulation of catalase activity [42] (see also below). It seems that the adipokinetic response is enhanced and or to some extent modified primarily in the presence of stress, caused by an injection or by an application of insecticides as in the two latter cases.

A lowering of the carbonyl contents also supports the suggestion of an operative role of AKHs in antioxidant action to counter OS in insects. The complex strategy of this action is still unclear; however, it is quite possible that more players, besides GSH, are involved. This suggestion is supported by measurement of the total antioxidant activity in cell-free plasma of *P. apterus* against Trolox (an analog of vitamin E) standards. This procedure relies on antioxidant activity of low-molecular-weight compounds. The results show that paraquat injection alone potentiates an antioxidant response that is significantly enhanced upon coinjection of paraquat with AKH. However, AKH injection alone is not capable of inducing antioxidant activity to the levels produced by paraquat alone or by coinjection with paraquat, although it is more enhanced compared to control groups [41]. Hence, this supports the suggestion that there must be some other additional mechanism(s) by which AKH acts to enhance antioxidant response.

The list of AKH activities involved in the OS control also includes the modulation of activity of catalase that is responsible for decomposition of toxic hydrogen peroxide into water and oxygen [18]. Application of insecticides endosulfan and malathion causes a significant increase in catalase activity in the whole body of *P. apterus* but when coapplied with AKH a significant decrease is recorded compared with insecticides alone. The catalase activity after the coapplication is still significantly higher than that after AKH treatment alone; however, the fall as compared to application of the insecticides is substantial, achieving 2.3- to 3.7-fold lower values. Since free radical-scavenging enzyme complexes like superoxide dismutase, catalase, and glutathione peroxidase are in the first line of cellular defense against oxidative injury [58–60], the induction of catalase after insecticide application is not surprising. Possibly, AKH reduces the production of hydrogen peroxide by an unknown mechanism, and therefore the activity of catalase necessary for reduction of hydrogen peroxide is lower.

AKH activities in the stress elicited by insecticides are also linked to one interesting phenomenon: an enhancement of the insecticide activity by AKH coapplication. This phenomenon is probably not directly connected with OS, because it was first reported for the insecticide permethrin [51], which does not possess the ability to generate OS, but a certain relationship to OS cannot be completely excluded when endosulfan or malathion, known to promote formation of free radicals, is used [61–63]. The coapplication of the insecticides with AKH increases *P. apterus* mortality up to 3-fold [42]. Almost nothing is known about the mechanism of the phenomenon, but it has been suggested that the AKH stimulation of metabolism [33] could enhance the insecticide action. This suggestion is supported by the AKH-induced significant increase of carbon dioxide production [42,51]—an indicator of metabolic rate in the experimental bugs. The increased metabolic rate could intensify the insecticide action by faster penetration of the insecticide into tissues and by a more intensive exchange of metabolites affecting the biochemical pathways, including also the OS reactions. However, in the absence of direct data this statement remains a speculation.

18.8 ROLE OF OTHER INSECT HORMONES IN OXIDATIVE STRESS

Not only have AKHs been implicated to be involved in hormonal control of antioxidative protective reactions in insects, but other hormones such as glucagon, ecdysteroids and JHs have also been suggested to be involved.

Glucagon: It is a 29-amino acid peptide well-known as a vertebrate hyperglycemic hormone. In insects, immunochemically similar glucagon-like peptides were reported in hemolymph [64], midgut [65, 66], and nervous system [66–69] of several insect species. On the other hand, a role for the glucagon-like peptides in insect body has not been explained satisfactorily. They could play a role in the brain-gut axis [70] and participate also in the control of digestive or other metabolic processes, but direct evidence is still missing. There are some indications that glucagon-like peptides play a role in regulation of insect glycemia, as was shown in the honey bee *Apis mellifera* [71], but this effect is most likely not common in other insects [72]. Analogous to vertebrates [55,73], Alquicer et al. [66] suggested for insects that glucagon could play a role in defense against OS, because injection of porcine glucagon into the *P. apterus* body elicits the antioxidant response by significantly increasing GSH and decreasing protein carbonyl levels in hemolymph, and decreasing both protein carbonyl and protein nitrotyrosine levels in CNS. Moreover, similarly as reported for the AKHs (see Section 18.7 for details), when coinjected with paraquat, glucagon partially eliminates OS markers elicited by this oxidative stressor and returns them to the control levels. Nevertheless, the lack of significant changes of AKH titer in *P. apterus* body after the injection of glucagon suggests that glucagon action is AKH independent.

Ecdysteroids: These are well-known insect steroid hormones playing a crucial role in insect developmental and reproductive events (see also Section 18.6). In vertebrates steroid hormones, namely, estrogens and related compounds possessing a phenolic A-ring, were shown to be involved in the control of OS. They inhibit the oxidation of cholesterol and the peroxidation of polyunsaturated fatty acids (diene conjugation or malondialdehyde formation) in the lipoproteins, microsomes, and other components of biological systems [74]. 20-Hydroxyecdysone, one of the most important member of insect steroid hormones, shows similar features. It has been proven that 20-hydroxyecdysone is a potent antioxidant able to minimize the OS impact of paraquat to *P. apterus* [17]. This ecdysteroid restrains lipid peroxidation and the formation of protein carbonyls, ameliorates changes in microsomal membrane fluidity, enhances the level of reduced glutathione, and upregulates the activity of γ-glutamyl transpeptidase in the brain. However, the protective effect of 20-hydroxyecdysone against the OS has also organismal dimensions. Certain hemolymph proteins, very sensitive to the paraquat treatment, are consistently present in the bugs being injected with 20-hydroxyecdysone despite the paraquat presence. The same injection ameliorates the suppressive paraquat effect on female fertility and in both sexes improves the survival rate curtailed by paraquat.

A similar function of 20-hydroxyecdysone is reported by Roesijadi et al. [75], who show that ecdysone induced methionine sulfoxide reductase A in the fruit fly *Drosophila melanogaster* is associated with enhanced resistance to hydrogen peroxide. Methionine residues in proteins are susceptible to oxidation when subjected to reactive oxygen or nitrogen species [76]. The reduction of the oxidized methionine form is catalyzed by methionine sulfoxide reductase [77]. Expression of the enzyme is regulated via the ecdysone receptor (EcR-UPS) complex [78] controlled by ecdysone. Overexpression of the enzyme is associated with enhanced protection against OS, while its knockdown results in hypersensitivity to OS [79, 80].

The mechanisms of action of these effects are unknown, but their existence indicates the importance of ecdysteroids for the management of OS in the insect body [17].

Juvenile hormones: These are insect terpenoid hormones with two main functions in insect life. As developmental hormones, they prevent premature initiation of insect metamorphosis in juvenile stadia and support reproduction by hormonally controlled gene expression of vitellogenins in adult females. Their role in OS seems to be indirectly mediated through the regulation of biologically active proteins like vitellogenins [81] and/or transferrin [82, 83].

Vitellogenins primarily serve as energetic and building components for a developing embryo in the insect egg. They are synthesized in specialized cells in the fat body or rarely in ovariols by a complicated hormonally controlled process (see 84) in which the JHs in most insect species play a crucial role. In the bee worker vitellogenins protect the organism against the oxidative damage of paraquat [81]. The results of the authors show that vitellogenins are a preferred target of oxidative carbonylation in comparison with other hemolymph proteins, which is a property that is indicative of antioxidant function [85]. There is a direct implication of this finding for bee aging: Treatment of low- and high-vitellogenin level bee phenotypes with paraquat showed that survival was significantly lower for the former group. The data show that vitellogenin activity is causally linked to OS resistance of the bee, and further, they can explain why vitellogenins are synthesized at high levels in honeybee queens and are abundant in long-lived workers [81].

Insect transferrin is known as an iron transporter, regulated by vitellogenin and JH activities. Its essential function is transport of iron that is required for a wide variety of metabolic processes including oxygen transport and electron transfer; iron also participates in the Fenton reaction (see Section 18. 2), in which the very reactive hydroxyl radical is generated [86]. Insect transferrin is regulated by a lot of diverse processes (87) including hormonal processes. As transferrin is suggested to be a vitellogenic protein, an involvement of the JHs is not surprising. Jamroz et al. [82] showed a strong suppression of the transferrin gene in the fat body by JH treatment of the cockroach *Blaberus discoidalis*. Harizanova et al. [83] demonstrated in the mosquito *Aedes aegypti* that the JH analog methoprene suppresses the increase of transferrin message after blood feeding. The reasons for the suppression are not properly understood: Transferrin gene could be a target of transcription factors of ecdysteroid-induced mosquito homologues, and a negative effect of JH on them could play some role [83].

18.9 CONCLUSION

The past couple of decades have yielded a plethora of literature advancing our understanding of the signal transduction pathways in response to oxidative stress. While these are important in mediating transcriptional response to oxidants at the molecular level, major players in stress response pathways such as stress-responsive hormones are only beginning to be implicated especially in insects. With this has also come an appreciation for the complexity of the endocrine-mediated responses and the awareness that these hormones may not be acting in isolation but intersecting with one another to mediate a myriad of the physiological and adaptive responses to oxidative stress in dose- and cellular context-dependent manners. Emerging from the survey of endocrine actions in stress responses (particularly oxidative stress) is a picture of extraordinary diversity and complexity, whether viewed in terms of the target cells, the metabolic pathways, or the physiological functions that the neurohormones regulate. Although we have tried not to be too elaborate, within our limited goal, we have discussed to the best of our available knowledge the role of insect stress hormones in oxidative stress. Molecular understanding of the interactions between the endocrine system and oxidative stress will continue to yield important insights into mechanisms that trigger such responses. We hope that the present review will stimulate further research within a framework of insect hormone actions to oxidative stress constituting a coherent, albeit complex and heterogeneous, physiological whole.

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