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REVIEW

MULTIFUNCTIONALITY OF ANTIOXIDANT SYSTEM IN INSECTS

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Abstract - Amount of reactive oxygen species (ROS) produced in tissues of vertebrates is regulated by their elimination through coordinate action and level of enzymatic (SODs - superoxide dismutases, CAT-catalase, GSH-Px - glutathione peroxidase, GSTs - glutathione-S-transferases and GR - glutathione reductase) and non-enzymatic (glutahione, ascorbate and tocopherols) antioxidant (AO) components. Different from vertebrates, incest species lack selenium-dependent GSH-Px and posses active enzymatic ascorbate recycling pathway serving to eliminate hydrogen peroxide. Moreover, the sites of ROS generation in insect cells are compartmentalized and dislocated within the cells. Consequently, AO components are widely distributed at subcellular level. The mode of the regulation of AO components functioning in insects has been studied in diverse insect species employing different agents acting as inducers of oxidative stress, as well as during reproduction, development and ageing. The results reported so far showed that incest AO system is very flexible and dynamically organized. Besides, it has been demonstrated that this system operates not only to eliminate extensive ROS but also to recompose particular ROS which express direct physiological effects (*e.g.* hydrogen peroxide and nitric oxide) and to establish optimal redox state.

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INTRODUCTION

Generation of reactive oxygen species (ROS) represents one of the accompanying phenomena during oxygen metabolism in aerobes. ROS are produced in diverse biochemical reactions and the respiration process during which about 2% of molecular oxygen are permanently transformed into superoxide anion radical ($O_2^{\cdot}$) represents the principal generator of ROS. On the other hand, superoxide anion radical is catalytically reduced to a high extent into hydrogen peroxide (H_2O_2) (Harman 1972; Nohl and Hegner 1978; Chance *et al.* 1979). Among other processes known to generate ROS in the concentrations significant for the cells and characteristic for insect species, it is worth mentioning monooxygenation of different classes of molecules($O_2^{\cdot}$ is generated), microsomal oxidase reactions (H_2O_2 is produced) (Hoffman and Hetru 1983), as well as Fenton and Haber-Weiss reactions resulting in hydroxyl radical (·OH) production (Halliwell and Gutteridge 1999). Because of their relatively high reactivity, ROS are interacting not only with each other but also absolutely unspecifically with different classes of the molecules within a cell. Hydroxyl radical, known for its high reactivity interacts with almost any of the vicinal molecules. Specificity of intracellular ROS action can lead in the end to the alterations in the structure of constitutive cellular molecules through a series of chain radical reactions. These structural alterations are termed oxidative injuries (Halliwell and Gutteridge 1999).

Connected to the above mentioned facts, a group of enzymes and low-molecular weight components that act transforming ROS into non-radical or weakly reactive molecular species has been classified as cellular **antioxidant defence mechanism.** Principal physiological role of this mechanism is to prevent development of oxidative injuries (Cotgreave *et al.* 1988; Matsuo 1993).

REACTIVE OXYGEN SPECIES (ROS) AND THE COMPONENTS OF ANTIOXIDANT SYSTEM IN INSECTS

The concept of antioxidant defence system suggested a functional connection of its components and completely viewed, it should be considered as a physiological system. This is justified by the fact that the sequences which involve components of antioxidant defence system and lead to the elimination of possible negative ROS effects are linked with each other. Dismutation of superoxide anion radical in the inner mitochondrial membrane to H_2O_2 by manganese-containing superoxide dismutase would be the first functional step as shown by the equation (1):

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{\text{MnSOD}} H_2O_2 + O_2 \qquad (1)$$

Cytosol copper-zinc-containing SOD form (CuZnSOD) dismutates $O_2^{\cdot-}$ in the cytosol also into H_2O_2 (Fridovich 1995). However, this reaction does not prevent further possible oxidative damages because H_2O_2 itself, represents a strong oxidative agent and a mediator of ·OH generation *via* Fenton and/or Haber-Weiss reactions. Hydrogen peroxide is enzymatically decomposed by the action of catalase - CAT (Halliwell and Gutteridge 1985):

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2 \qquad (2)$$

In vertebrates, H_2O_2 can also be transformed by the action of selenium-dependent glutathione peroxidase - GSH-Px (Cotgreave *et al.* 1988):

$$2GSH + H_2O_2 \xrightarrow{\text{GSH-Px}} 2H_2O + GSSG \qquad (3)$$

However, the data reported so far demonstrated the absence of this enzyme in insect species (Smith and Shrift 1979; Sohal *et al.* 1990; Bolter and Chefurka 1990; Ahmad 1992; Grubor-Lajšić *et al.* 1996; Jovanović-Galović *et al.* 1997; Jovanović-Galović 1998). Due to its higher affinity for H_2O_2 comparing to CAT, GSH-Px is considered to play a key role in the maintenance of the cellular homeostasis in mammalian tissues. Since insects do not contain this important functional component, one can ask a question on the organization of protection against oxidative injuries in insect species. However, in 1995, Felton and Summers reported the activity of a specific ascorbate-recycling mode for hydrogen peroxide elimination to be present in insects. Reduction of H_2O_2 by the action of ascorbate peroxidase - APOx, representing the first step, as shown by the equation (4):

$$AsA + H_2O_2 \xrightarrow{\text{APOx}} 2H_2O + DAsA \qquad (4)$$

The resulting dehydroascorbate (DAsA) gets further retransformed into the ascorbate by the action of dehydroascorbate reductase DHAR (Summers and Felton 1993), as seen from the equation (5):

$$2GSH + DAsA \xrightarrow{\text{DHAR}} AsA + GSSG \qquad (5)$$

Glutathione (GSH) serves as an electron donor for this reaction during which it is oxidized to its disulfide form GSSG. Reduction of thus formed GSSG occurs by the action of glutathione reductase - GR and NADPH consumption (Griffith 1980) as shown by the following equation:

$$GSSG + 2NADPH \xrightarrow{\text{GR}} 2GSH + NADP^+ \qquad (6)$$

Such a mode of protection against oxidative injuries is rather functional and represents the first line of defence at the cost of consumption of the reduction cofactors.

Taking into account the absence of GSH-Px activity in insects on the one hand and considering functional efficiency of an antioxidant system outlined in such a way on the other hand, it could be assumed as theoretically completed and functionally sufficient, because the above listed reactions result in the formation of water and oxygen molecules. However, Fenton reaction and/or Haber-Weiss reaction can lead to the fomation of hydoxyl radical (·OH) which can further produce lipid peroxides *via* its chain reactions with lipids. Because of that, the enzymes catalyzing decomposition of

lipid peroxides represent also the components of the antioxidant system. In the cells of vertebrate species lipid peroxides are transformed into the corresponding alcohols by the action of selenium-dependent glutathione peroxidase and a glutathione-S-transferase (GST) isoform possessing peroxidase activity - GPOx. In insect species, however, lipid peroxides are eliminated only by the action of GPOx because of already mentioned fact that they do not possess the activity of selenium-dependent GSH-Px (Ahmad *et al.* 1989):

$$2GSH + LOOH \xrightarrow{GPOx} LOH + H_2O + SSG \qquad (7)$$

It could be considered that antioxidant components listed above provide an adequate protection at the level of basal metabolism and decrease the possibility for the development of cellular injuries. However, there is no enzymatic protection for the elimination of ·OH radical. It is eliminated by ascorbic acid (AsA) in hydrosol and by vitamin E in lipophilic compartments, the latter being dominant in ceasing radical chain reactions in the cell membrane, thus preventing the evolvement of lipid peroxides (Halliwel and Gutteridge 1999). As a result of the reactions of ascorbate and vitamin E with radical species, stable ascorbate and vitamin E radicals arise and it can be assumed that this arrests further radical propagation. In insects, the activity of ascorbate radical reductase (ARR) enables the return to zero homeostatic state (Felton and Duffey 1992) as shown by the following equation:

$$AsA^{\cdot} + NADPH \xrightarrow{ARR} AsA + NADP \qquad (8)$$

The data of several authors clearly demonstrated that ascorbic acid acts efficiently and at a high reaction rate reducing vitamin E radical while transforming itself to the corresponding radical form which is further enzymatically reduced to AsA as shown by the equation (8) (Niki 1991; Wijesundara and Berger 1994) In this way the concept on AO system in insect species could be assumed to be completed. However, the number of antioxidant components and their functional role in insects still remains an open question. The presence of different peroxidase (POx) classes is one of controversies. In the reaction of hydrogen peroxide reduction, peroxidases generally use different classes of reduction cofactors (RH_2):

$$RH_2 + H_2O_2 \xrightarrow{POx} R + 2H_2O \qquad (9)$$

However, peroxidases have been detected neither in all insect species studied so far, nor in all insect tissues (Ahmad 1992; Felton and Summers 1995). So, they don't have to be assumed as an obligate component of antioxidant protection in insects.

Different classes of generically and/or nutritionally present non-protein molecules have been considered for a relatively long period of time as to play a role of antioxidants in insects. This list includes carotenes (Robinson and Beatson 1985; Ahmad 1992), carotenoids (Kayser 1982), uric acid (Hilliker *et al.* 1992; Felton and Summers 1995), polyols and some carbohydrates (Halliwell and Gutteridge 1990). However, it is still unclear whether the antioxidant action represents their primary role or the consequence of their chemical properties and relatively high concentration within the tissues under certain physiological conditions or developmental stage (Grubor-Lajšić *et al.* 1992a; Worland *et al.* 1998, 2000). However, it has been demonstrated that trehalose, a primary carbohydrate in the insect haemolymph can scavenge hydroxyl radical much more efficiently than the glucose (Felton and Summers 1995). Glycerol and ethylene glycol accumulating during the winter period in insect species as anhydroprotectors and antifreeze compounds have also been suggested to be potential free radical scavengers (Block 1990; Duman *et al.* 1991; Block *et al.* 1993). In addition, it should be mentioned that 3-hydroxykynurenine, a precursor of the eye pigment in insects, also expresses antioxidant properties (Wada no *et al.* 1993).

It is also very interesting to discuss the formation of peritrophic matrix - PM (peritrophic membrane) in insects. It represents a special protective layer formed around ingested material in the midgut of most of insect species. PM protects epithelial midgut cells against abrasion by food particles, binds toxicants and prevents penetration of pathogenes into epithelial midgut cells. It consists of chitin and protein layer that represents the first barrier against prooxidants. Polysaccharide prope-rties of chitin help it to serve as an efficient sink of reactive oxygen species. It has been reported that under *in vitro* conditions, PM not only diminishes the extent of lipid peroxidation, but also represents a rather efficient scavenger of OH radicals (Felton and Summers 1995). PM greatly resembles mammalian gastric mucin, which is also a polysaccharide-protein complex that acts as an important scavenger of reactive oxygen species.

There are also some molecules which are not antioxidants but act diminishing potential danger of oxidative injuries and because of that they are very frequently referred to as a part of antioxidant defence. Transferrin and ferritin belong to this group of compounds, although their principal physiological role is to transport and storage iron and copper (Locke and Nichol 1992). The reason for their involvement into the concept of antioxidant system should be sought for in the elimination of free metal ions that could lead to both the initiation and propagation of radical processes from a broad cellular environment and cytosol into cell compartments. In addition to transferrin and ferritin, cerulloplasmin that catalyzes the oxidation of Fe^{2+} to Fe^{3+} without ROS generation and metallothioneins and similar compounds belong to the above group of molecules that efficiently bind the metals (Maroni *et al.* 1986). However, their active participation in the process of antioxidant defence and thus in the antioxidant system of insects still remains to be elucidated (Polek *et al.* 1993). Connected to the above assumptions, low molecular weight compounds such as ATP, citrate, lactate, uric acid, *etc.* which behave as chelating agents, could be hypothesized to play a role in antioxidant defence, as well. When considering whether all these molecules represent components of the antioxidant system, it is of utmost importance to establish their relation to the sum and significance of radical processes themselves, and especially their relation to primary antioxidant components. Further studies along these lines are necessary to determine experimentally these relations.

COMPARTMENTATION OF GENERATION SITES OF ROS AND ANTIOXIDANT SYSTEM COMPONENTS IN INSECTS

Subcellular distribution of AO components in insects clearly differs from that occurring in vertebrates. The most prominent difference is related to catalase which is widely distributed at subcellular level in insects (Ahmad and Pardini 1990), while in mammalian species this enzyme is dominantly localized in peroxisomes (Fukami and Flatmark 1986). Such a wide subcellular distribution of catalase in insect species could be explained in terms of the absence of selenium-dependent glutathione peroxidase. Also, ferritin is dominantly localized in vacuoles (Nichol and Locke 1989) and in this way it is distant from cytoplasmic membranes, cytosol and the nucleus what minimizes the possibility for the generation of ·OH radicals and lipid peroxides. It seems that the distribution of GPOx activity is accompanying the sites of potential generation of lipid peroxides, while in mitochondria the activity of this enzyme has not been detected (Ahmad *et al.* 1988; Ahmad and Pardini 1990).

The level of insect antioxidant system components was found to be tissue-specific. Metabolically active tissues such as fat body, midgut and Malpighian tubules, known for the most extensive ROS production possess relatively significant activity of all AO enzymes. In contrast, the activity of AO enzymes in haemolymph is either very low or even under the level of detectability (Ahmad 1992), although it contains a protein complex that actively generates $O_2^{\cdot}$ (Toru 1994). Production of free radical species in insect haemolymph has been confirmed by the data of numerous authors (Arakawa 1995; Arakawa *et al.* 1996; Grubor-Lajšić 1997b; Whitten and Ratcliffe 1999). Since both oxidants and antioxidants take part in the processes of differentiation, signal transduction, gene expression and apoptosis, it could be speculated that an increased radical production might be important for histolysis and histogenesis processes occurring during metamorphosis of insects (Sohal *et al.* 1986; Sohal and Allen 1990).

FUNCTIONAL ASSOCIATION AND COORDINATION OF ANTIOXIDANT SYSTEM COMPONENTS IN INSECTS

The problem of AO defence has been usually discussed in the available literature taking into consideration enzymatic components (Pritsos *et al.* 1988 a,b; Ahmad and Pardini 1990; Pritsos 1990; Felton and Duffey 1991, 1992; Summers and Felton 1993; Batcabe *et al.* 1994). This is relatively logical, because these components represent constitutive elements of the endogenous system what makes it possible to endogenously regulate their activity. Concentration of low-molecular weight antioxidants within the tissues is susceptible to diet-related modifications. Thus, the regulation of their level within the frame of a general concept of the antioxidant system represents the objective of more complex mechanisms which include trophic regulation, as well as processes of adaptation and development, ecological interactions and coevolution. However, due to the heterogeneity of nutritive and environmental conditions numerous insect species are exposed to, there are some exceptions. The number of antioxidants contained in plant diet is rather high and all of them can play a role of an additional antioxidant defence that disguises possible role of the primary, enzymatic antioxidants. Also, coevolution of insects and host plants resulted in the development of trophic adaptations and as a consequence, this led to

differences in utilization of plant nutrient components which can contribute to the establishment of oxidative equilibrium in a different way. The present study was aimed at better understanding of endogenous functional organization of antioxidant system characterizing insect species. Although the differences in the organization (relative share and significance of individual components) result from genetic programmes characteristic for individual species, metabolic rate and combination of different genetic factors in broader sense of meaning, and although it seems that the animals maintain homeostasis by the entire antioxidant level (Cutler 1984; Sohal and Allen 1986), the problem should be considered from the aspect of their structural organization, as well.

Analysis of ROS generation pathways clearly shows that the simplest way for the understanding of the AO system functionality could be found when considering physiological axis SOD → CAT. So, the entire generated $O_2^{\cdot-}$ would be dismutated by the action of SOD to H_2O_2, which would be further transformed into water molecules by catalase action. Because of that, these two enzymes were assumed to be a physiological tandem. Allen *et al.* (1984) treated houseflies (*Musca domestica*) with a metabolic generator of $O_2^{\cdot-}$ and observed not only an extensive generation of H_2O_2 as a result of dismutation, but also an increased CAT activity. This means that the activities of these two enzymes should be coupled and in correlation with the resulting reduced concentration of oxidatively damaged cellular molecules. Such an assumption has been confirmed by the results of sophisticated experiments performed by Orr and Sohal (1994). These authors have inserted extracopies of the corresponding genes for SOD and CAT in *Drosophila melanogaster* genome and observed a decreased amount of oxidative damages in transgenic individuals comparing to that recorded in the original wild type controls (Orr and Sohal 1994; Sohal *et al.* 1995). From the onset of experiments, they included only modified fruit flies which had SOD and CAT activity increased by 50%, while all other modified transgenic genomes were excluded. This enabled them to use only individuals with an increased gene expression, so that the enhanced enzymatic activities were not induced but constitutive ones. The results of these experiments confirmed only the theoretical concept, but not the physiological one which would mean an active correlation of the components as a dynamic response to the changes of the oxidative status. Such a correlation has been recorded in mammals but as SOD → GSH-Px metabolic axis (Blagojević *et al.* 1998). At the same time, in bean weevil (*Acanthoscelides obtectus* Say) as a representative of insect species, no correlation between the activities of SOD and CAT has been recorded (Blagojević *et al.* 1998). Experiments with transgenic fruit flies with inserted individual SOD gene failed to provide the expected effects (Seto *et al.* 1990). Even a higher mortality rate has been observed in transgenic *Drosophila* individuals with a SOD extra gene copy in relation to the corresponding controls (Reveillaud *et al.* 1991) and in this context, hypothesis on SOD-CAT as a physiological tandem would be justified. However, higher SOD activity in transgenic fruit flies was not accompanied by an endogenous increase of CAT activity necessary to establish homeostasis according to such a model (Reveillaud *et al.* 1992). Also, the results of experiments with the reduction of the constitutive SOD and CAT activities revealed no positive coordination and correlation of SOD and CAT activity in both directions (Reveillaud *et al.* 1992; Orr *et al.* 1992). The data obtained in the same experiments even demonstrated that neither SOD, nor CAT are essential for the cellular viability *per se* (Philips *et al.* 1989; Orr *et al.* 1992). This means that insects either have a broader tolerance limit against ROS than the vertebrates, or antioxidant system plays another physiological role in addition to the coupled protective one. In the latter case, antioxidant system in insects should be examined in more details and from different aspects.

Principles of the regulation of ROS equilibrium, functional connections and possible coordination of AO system components in insects were analyzed under conditions of experimentally provoked oxidative stress employing different agents. A general conclusion that could be drawn out from thus obtained experimental results suggests that depending on the kind of oxidative stress, AO system dynamically establishes new relations that provide homeostasis. These new relations also include dynamic reorganization of isoforms of AO components as suggested recently by Perić-Mataruga *et al.* (2000). The data obtained in such experiments demonstrated that the role of SOD and CAT activity is significant, but not the only one. An increased SOD activity under conditions of oxidative stress has not been always accompanied by an enhanced CAT activity, although the results have clearly demonstrated an elevated H_2O_2 production (Perić-Mataruga *et al.* 1997). Numerous authors recorded an increased glutathione level as a response to oxidative stress applied to several insect species (Allen *et al.* 1984, 1985; Sohal *et al.* 1984; Sohal 1988; Perić-Mataruga *et al.* 1997), as well as the activation of the mechanism directed towards O_2 consumption (Allen

and Sohal 1982; Sohal *et al.* 1984; Allen *et al.* 1984, 1985). Taking into account the absence of GSH-Px in insects, an increased glutathione levels could be explained in terms of its direct antioxidant properties and the role of cofactors in GST-mediated elimination of lipid peroxides, but also by the restitution of SH groups of active molecules, maintenance of the redox state within a cell and control of cellular Ca^{2+} ion concentration. Participation of GSH in ascorbate recycling mechanism has not been examined in the above experiments. However, keeping in mind the role of ascorbate recycling enzymes in insects, it is clear that this mechanism can be of great importance from physiological point of view. Connected to that, it is worth mentioning that the ascorbate-enriched diet was shown to lead to a decrease of glutathione level in insect cells (Sohal *et al.* 1985), clearly pointing to an economical cellular manipulation with redox-active molecules. It is interesting to note that in the cases of an exceeding working limit of constitutive AO enzymes, a compensatory decrease of O_2 consumption occurs. This demonstrates two-way functional connection of oxidative metabolism level and the amount of ROS. It could mean that ROS mediate or take part in the processes of metabolism regulation and if affirmative, the role of AO system would be to discretely direct their concentrations towards the regulatory ones. There are some data in the available literature demonstrating a direct regulatory role of ROS in physiological processes of insects. It has been shown recently that H_2O_2 can provoke apoptosis of insect cells (Hasnain *et al.* 1999), as well as the sequence of molecular events of induction characteristics (Courgeon *et al.* 1988). To obtain a complete picture on a possible regulatory role of AO system which could be of great significance for insect physiology, it is necessary to provide an insight into the functioning of this system not only under conditions of protection against oxidative injuries but also under conditions of the regulation of the basal vital processes.

Most of the results related to the functioning of insect AO system have been obtained using different insect species as experimental models in the studies concentrated on ageing processes and especially on the evaluation of free-radical theory of ageing. Quite a number of papers referred to in the present review had such a starting point. It has been believed that the ageing represents a process of accumulation of ROS-mediated oxidative injuries and that AO system plays a key role in the longevity. The results obtained in experiments of Orr and Sohal (1994) confirmed such a hypothesis. However, numerous authors failed to demonstrate that longevity or increased average life span are accompanied by a clear linear elevation of AO activity (Massie *et al.* 1975; Niedzwiecki *et al.* 1992; Šešlija *et al.* 1999). An increased average life span can be achieved in insects employing different selection regimes which act altering physiological status of individuals, as well. So, it has been reported that *Drosophila melanogster* individuals genetically manipulated for postponed senescence employing selection regime for resistance to starvation had increased lipid levels (Service 1987; Zwaan *et al.* 1991). On the other hand, application of the selection regime directed towards resistance against desiccation (Service *et al.* 1985; Rose. 1990) provided individuals with an elevated glycogen concentration accompanied by a decreased motoric activity and metabolic rate (Graves *et al.* 1992). However, there is no either biochemical and/or physiological ageing pattern that could be positively established and reasonably presented (Sohal 1985). Besides, longevity is a quantitative character (Luckinbill *et al.* 1988; Arking *et al.* 1993) controlled by several gene loci (Hutchinson and Rose 1990; Luckinbill *et al.* 1987). So, keeping these facts in mind, AO system could be described as a segment of functional metabolic control of vital processes.

Changes in insect AO system components have been recorded during development (Grubor-Lajšić *et al.* 1992b, 1997a; Perić-Mataruga *et al.* 1997, 2000; Jovanović-Galović 1998), as well as in reproduction processes (Blagojević 1996; Blagojević *et al.* 1997; Šešlija *et al.* 1999) and the results did not demonstrate that the participation of AO components is connected only with the segment of antioxidant defence. So, Grubor-Lajšić *et al.* (1997a) observed a species-specific change in glutathione level in diapausal larvae of European corn borer (*Ostrinia nubilalis*) under conditions of a decreased ambient temperature. These results were interpreted in terms of developmental programme in which glutathione plays a very significant role unrelated to its antioxidant properties. In addition, it has been shown that both dehydroascorbate reductase and glutathione play an important role in developmental processes of *Ostrinia nubilalis*. At the same time, compensatory functioning of DHAR and GR in the direction of an increased reduction of cofactors, ascorbate or glutathione, available to the larvae or the pupae in a certain developmental stage to a greater extent, as well as a clearly correlated activity in the control of redox state were observed (Jovanović-Galović 1998). It is interesting to note that physiological

mechanisms during insect development represents the consequence of long-lasting coevolution of insects and host plants, ecological factors and inherent developmental programme. The results obtained in experiments related to insect reproduction pointed to catalase and glutathione as the factors involved in this process (Blagojević *et al.* 1997). Reproduction of insects includes signal, hormone-mediated events and metabolic activity. From this point of view, AO system could be clearly classified as a segment of protection. However, it is known that in insects NO - cGMP signal pathway is operative (Ribeiro *et al.* 1993; Dow *et al.* 1994). Consequently, the role of AO system in these processes could not be assumed as to be passive, because a significant part of this system is NO redox-based (Lipton *et al.* 1993). Thus, the role of AO system is not limited to the neutralization of ROS, because it takes part in prestructuring of active molecules towards physiologically functional concentrations. Similar to vertebrates, active generation of free radical species has been demonstrated to occur in insect cells. Superoxide anion radical is actively generated in haemocytes of the haemolymph (Whitten and Ratcliffe 1999), while in addition to superoxide anion radical in neural cells of insects and salivary glands of some blood-sucking insect species, nitrogen oxide (NO) with a distinct physiological role is synthesized (Ewer *et al.* 1994; Dow *et al.* 1994; Ribeiro and Nussenzveig 1993). So, additional logics on the physiological role of antioxidant system components still remains and open question. This is related first of all to SOD as a barrier of peroxinitrite ($ONOO^-$) generation, the role of glutathione as a redox factor, mediator of different cellular processes and physiological carrier of S-nitrozothiol. In addition, reactions of ROS with regulatory molecules can result in the change of the effector physiological processes. It is to be expected that in the near future the studies along these lines will provide the answers to some of the above questions what would greatly enrich our understanding on multifunctional activity of individual antioxidant components in insects.

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REFERENCES

Ahmad, S. (1992). Biochemical defense of pro-oxidant plant allelochemicals by herbivorous insects. *Biochem. Syst. Ecol.* **20**, 269-296.

Ahmad, S., Beilstein, M.A. and *Pardini, R.S.* (1989). Glutathione peroxidase activity in insects: A reassessment. *Arch. Insect Biochem. Physiol.* **12**, 31-49.

Ahmad, S. and *Pardini, R.S.* (1990). Mechanisms for regulating oxygen toxicity in phytophagous insects. *Free Rad. Biol. Med.* **8**, 401-413.

Ahmad, S., Pritsos, C.A., Bowen, S.M., Heisler, C.R., Blomquist, G.J. and *Pardini, R.S.* (1988). Antioxidant enzymes of larvae of the cabbage looper moth, *Trichoplusia ni*: subcellular distribution and activities of superoxide dismutase, catalase and glutathione reductase. *Free Rad. Res. Comm.* **4**, 403-408.

Allen, R.G., Farmer, K.J. and *Sohal, R.S.* (1985). Effect of catalase inactivation on levels of inorganic peroxides, superoxide dismutase, glutathione, oxygen consumption and life span in adult houseflies (*Musca domestica*). *Biochem. J.* **216**, 503-506.

Allen, R.G., Farmer, K.J., Newton. R.K. and *Sohal. R.S.* (1984). Effects of paraquat administration on longevity, oxygen consumption, lipid peroxidation, superoxide dismutase, catalase, glutathione reductase, inorganic peroxides and glutathione in adult housefly. *Comp. Biochem. Physiol.* **78C**, 283-288.

Allen, R.G. and *Sohal, R.S.* (1982). Life-lenghtening effect of - radiation in the housefly, *Musca domestica*. *Mech. Ageing Dev.* **20**, 369-374.

Arakawa, T. (1995). Possible involvement of an enzymatic system for superoxide generation in lepidopteran larval haemo-lymph, *Arch. Insect. Biochem. Physiol.* **29**, 281-291.

Arakawa, T., Kato, Y., Hattori, M. and *Yamakawa, M.* (1996). Lipophorin - a carrier for lipids in insects participates in superoxide production in the haemolymph plasma. *Insect Biochem. Mol. Biol.* **26**, 403-409.

Arking, R., Dudas, S.P. and *Baker, G.T.* (1993). Genetic and environmental factors regulating the expression of an extended longevity phenotype in a long lived strain of *Drosophila*. *Genetica* **9**, 127-142.

Batcabe, J.P., MacGill, R.S., Zaman, K., Ahmad, S. and *Pardini, R.S.* (1994). Mitomycin C induced alterations in antioxidant enzyme levels in a model insect species, *Spodoptera eridania*. *Gen. Pharmacol.* **25**, 569-574.

Blagojević, D. (1996). The role of protection against oxidative damage in the ageing process of bean weevil (*Acanthoscelides obtectus*). *Ph.D. Thesis.* Faculty of Biology, University of Belgrade.

Blagojević, D., Buzadžić, B., Korać, B., Saičić, Z.S., Radojičić, R., Spasić, M.B. and *Petrović, V.M.* (1998). Seasonal changes in the antioxidative defence in ground squirrels (*Citellus citellus*): possible role of GSH-Px. *J. Environ. Pathol. Toxicol. Oncol.* **17**, 241-250.

Blagojević, D., Lazarević, J., Perić-Mataruga, V. and *Spasić, M.B.* (1997). Effects of reproductive activity on antioxidative defence of gypsy moth (*Lymantria dispar* L.). *Symp. Serb. Entomol. 97*, Go, Serbia, Book of Abstracts, p 17.

Block, W. (1990). Cold tolerance of insects and other arthropodes. *Phil. Trans. R. Soc. Lond.* **B 326**, 613-633.

Block, W., Grubor-Lajšić, G. and *Worland, R.* (1993). Cold tolerance of a larval tipulid from an upland habitat. *Cryo-Lett.* **14**, 185-192.

Bolter, C.J. and *Chefurka, W.* (1990). The effect of phosphine treatment on superoxide dismutase, catalase and peroxidase in the granary weevil, *Sitophilus granarius*. *Pest. Biochem. Physiol.* **36**, 52-60.

Chance, B., Sies, H. and *Boveris, A.* (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59,** 527-605.

Cotgreave, I.A., Moldeus, P. and *Orrenius, S.* (1988). Host biochemical defence mechanisms against prooxidants. *Ann. Rev. Pharmacol. Toxicol.* **28,** 189-212.

Courgeon, A., Rollet, E., Becker, J., Maisonhaute, C. and *Best-Belpomme, M.* (1988). Hydrogen peroxide (H_2O_2) induces actin and some heat-shock proteins in *Drosophila* cells. *Eur. J. Biochem.* **171,** 163-170.

Cutler, R.G. (1984). Antioxidants, aging and longevity. In: *Free Radicals in Biology,* **6,** 371-428, (Ed. W.A. Pryor), New York, Academic Press.

Dow, J.A.T., Maddrell, S.H.P., Davies, S., Skaer, N.J.V. and *Kaiser, K.* (1994). A novel role for the nitric oxide cGMP signaling pathway: the control of epithelial function in *Drosophila. Am. J. Physiol.* **266,** R1716-R1719.

Duman, J.G., Wu, D.W., Xu, L., Tursman, D. and *Olsen, T.M.* (1991). Adaptations of insects to subzero temperatures. *Quart. Rev. Biol.* **66,** 387-410.

Ewer, J., De Vente, J. and *Truman, J.W.* (1994). Neuropeptide induction of cyclic GMP increases in the insect CNS: resolution at the level of single identifiable neurons. *J. eurosci.* **14,** 7704-7712.

Felton, G.W. and *Duffey, S.S.* (1991). Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. *J. Chem. Ecol.* **9,** 1715-1732.

Felton, G.W. and *Duffey, S.S.* (1992). Ascorbate oxidation/reduction in *Helicoverpa zea* as a scavenging system against oxidative plant defenses. *J. Chem. Ecol.* **17,** 1715-1732.

Felton, G.W. and *Summers, C.B.* (1995). Antioxidant systems in insects. *Arch. Insect Biochem. Physiol.* **29,**187-197.

Fridovich, I. (1995). Superoxide and superoxide dismutases. *Ann. Rev. Biochem.* **64,** 97-112.

Fukami, M.H. and *Flatmark, T.* (1986). Studies on catalase compartmentation in digitonin-treated rat hepatocytes. *Biochim. Biophys. Acta* **889,** 91-94.

Graves, J.L., Toolson, E.C., Jeong, C., Vu, L.N. and *Rose, M.R.* (1992). Desiccation, flight, glycogen and postponed senescencein *Drosophila melanogaster. Physiol. Zool.* **65,** 268-286.

Griffith, O.W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinyl pyridine. *Anal. Biochem.* **106,** 207-212.

Grubor-Lajšić, G., Block, W., Jovanović, A. and *Worland, R.* (1996). Antioxidant enzymes in the Antarctic fly, *Belgica antarctica. Cryo-Lett.* **17,** 39-42.

Grubor-Lajšić, G., Block, W., Telesmanić, M., Jovanović, A., Stevanović, D. and *Baca, F.* (1997b). Effect of cold acclimation on the antioxidant defense system of two larval Lepidoptera (Noctuidae). *Arch. Insect Biochem. Physiol.* **36,** 1-10.

Grubor-Lajšić, G., Block, W. and *Worland, R.* (1992a). Comparison of the cold hardiness of two larval Lepido-ptera (Noctuidae). *Physiol. Entom.* **17,** 148-152.

Grubor-Lajšić, G., Jovanović-Galović, A., Taški, K. and *Vujović, G.* (1997a). Superoxide anion generation in larvae and pupae of the European corn borer *Ostrinia nubilalis,* Hubn. *Acta Entomol. Serb.* **2,** 137-140.

Grubor-Lajšić, G., Kovačević, R., Stevanović, D., Jovanović, A., Karaman, I., Matavulj, M. and *Sarač, M.* (1992b). Activity and isosymic forms of Cu, Zn-SOD in the hemolymph of diapausing and non-diapausing *Ostrinia nubilalis* and *Sesamia cretica* caterpillars. *Zborn. Mat. srpske prir. nauke* **83,** 19-25.

Halliwell, B. and *Gutteridge, J.M.C.* (1985). Free Radicals in Biology and Medicine. Oxford, Clarendon Press.

Halliwell, B. and *Gutteridge, J.M.C.* (1990). The antioxidants of human extracellular fluids. *Arch. Biochem. Biophys.* **280,** 1-8.

Halliwell, B. and *Gutteridge, J.M.C.* (1999). Free Radicals in Biology and Medicine. New York, Oxford University Press Inc.

Harman, D. (1972). The biological clock: The mitochondria? *J. Am. Geriat. Soc.* **20,** 145-147.

Hasnain, S.E., Taneja, T.K., Sah, N.K., Mohan, M., Pathak,, N., Sahdev, S., Athar, M., Totey, S.M. and *Begum, R.* (1999).. *In vitro* cultured *Spodoptera frugiperda* insect cells: Model for oxidative stress-induced apoptosis. *J. Biosci.* **24,** 13-19.

Hilliker, A.J., Duyf, B., Evans, D. and *Philips, J.P.* (1992). Urate-null rosy mutants of *Drosophila melanogaster* are hy-persensitive to oxygen stress. *Proc. Natl. Acad. Sci. USA* **89,** 4343-4347.

Hoffman, J.A. and *Hetru, C.* (1983). Ecdysone. In: *Endocrinology of Insects,* 65-88, (Eds. R.G.H. Downer and H. Laufer), New York, Alan R. Liss, Inc.

Hutchinson, E.W. and *Rose, M.R.* (1990). Quantitative genetic analysis of postponed aging in *Drosophila melanogaster.* In: *Genetic Effects on Aging II,* 57-63, (Ed. D.E. arrison), Caldwell, NJ, Telford.

Jovanović-Galović, A. (1998a). Antioxidant system of European corn borer (*Ostrinia nubilalis,* Hbn): subcellular distri-bution and the role of antioxidants in metamorphosis. *Ph.D. thesis,* University of Novi Sad.

Jovanović-Galović, A., Grubor-Lajšić, G., Taški, K. and Nikolić, Lj. (1998b). Activities of antioxidant enzymes in diapausing and nondiapausing larvae of *Ostrinia nubilalis* Hbn (Lepidoptera). 1st Intern. Conf. Chem. Soc. South-East Eur. Countries, Halkidiki, Greece, Book of Abstr. 2, PO 702.

Jovanović-Galović, A., Grubor-Lajšić, G., Taški, K. and *Vujović, G.* (1997). Subcellular distribution and activi-ties of antioxidant enzymes in the European corn borer (*Ostrinia nubilalis*) Hbn. *11th Balkan Biochem. Biophys. Days,* Abstr. 127, 393,Thessaloniki, Greece.

Kayser, H. (1982). Carotenoids in insects. In: *Carotenoids Chemistry and Biochemistry,* 195-210, (Eds G. Britton and T.W. Goodwin) Oxford, Pergamon Press.

Lipton, S.A., Choi, Y.B., Pan, H.Z., Lei, S.Z., Chen, H.V., Sucher, N.J., LoscalzoJ., Singel, D.J. and *Stamler, J.S.* (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* Lond. **364,** 636-631.

Locke, M. and *Nichol, H.* (1992). Iron economy in insects: transport, metabolism, and storage. *Annu. Rev. Entomol.* **37,** 195-215.

Luckinbill, L.S., Clare, M.J., Krell, W.L., Cirocco, W.C. and *Richards, P.A.* (1987). Estimating the number of genetic elements that defer senescence in *Drosophila. Evol. Ecol.* **2,** 85-94.

Luckinbill, L.S., Graves, J.L., Reed, A.H. and *Koetsawang, S.* (1988). Localizing genes that defer senescence in *Drosophila melanogaster. Heredity* **60,** 367- 374.

Maroni, G., Lastowski-Perry, D., Otto, E. and *Watson, D.* (1986). Effect of heavy metals on *Drosophila* larvae and metallothionein cDNA. *Environ. Health Perspect.* **65,** 107-116.

Massie, H.R., Baird, M.B. and *McMahon, M.M.* (1975). Loss of mitochondrial DNA with aging. *Gerontology* **21,** 231-237.

Matsuo, M. (1993). *Free Radicals and Aging,* (Ed. P.Y. Byung), London, C.R.C. Press, Boca Raton.

Nichol, H. and *Locke, M.* (1989). The characterization of ferritin in an insect. *Insect Biochem.* **6,** 587-602.

Niedzwiecki, A., Reveillaud, L. and *Fleming, J.F.* (1992). Changes in superoxide dismutase and catalase in aging heat shocked *Drosophila. Free Rad. Res. Comm.* **17,** 355-367.

Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr.* **54,** 1119S-1124S.

Nohl, H. and *Hegner, D.* (1978). Do mitochondria produce oxygen radicals *in vivo? Eur. J. Biochem.* **82,** 863-867.

Orr, W.C., Arnold, L.A. and *Sohal, R.S.* (1992). Relationship between catalase activity, life span and some parameters associated with antioxidant defenses in *Drosophila melanogaster. Mech. Ageing Dev.* **63,** 287-296.

Orr, W.C. and *Sohal, R.S.* (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster. Science* **263,** 1128-1130.

Perić-Mataruga, V., Blagojević, D., Lazarević, J. and *Spasić, M.B.* (2000). Host plant effects on the presence of superoxide dismutase isoforms in gypsy moth midgut. *Biologica* **55,** 527-533, Bratislava.

Perić-Mataruga, V., Blagojević, D., Spasić, M.B., Ivanović, J. and *Janković-Hladni, M.* (1997). Effect of the host-plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins. *J. Insect Physiol.* **43,** 101-106.

Philips, J.P., Campbell, S.D., Michaud, D., Charbonneau, M. and *Hilliker, A.J.* (1989). Null mutation of copper/zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. *Proc. Natl. Acad. Sci. USA* **86,** 2761-2765.

Polek, B., Godocikova, J., Batora, R. and *Ursinyova, M.* (1993). Cu-binding proteins of digestive tract of *Galleria mellonella* caterpillars (Lepidoptera: Pyralide). *Biologica* **48,** 631-636, Bratislava.

Pritsos, C.A. (1990). Antioxidant enzyme level response to prooxidant allelochemicals in larvae of the southern armyworm moth, *Spodoptera eridania. Free Rad. Res. Comm.* **9,**127-133.

Pritsos, C.A., Ahmad, S., Bowen, S.M., Blomquist, G.J. and *Pardini, R.S.* (1988b). Antioxidant enzyme activities in the southern armyworm, *Spodoptera eridania. Comp. Biochem. Physiol.* **90C,** 423-427.

Pritsos, C.A., Ahmad, S., Bowen, S.M., Elliott, A.J., Blomquist, G.J. and *Pardini, R.S.* (1988a). Antioxidant enzymes of the black swallowtail butterfly, *Papilio polyxenes* and their response to the prooxidant allelochemical, quercetin. *Arch. Insect Biochem. Physiol.* **8,** 101-112.

Reveillaud, I., Kongpachith, A., Park, R. and *Fleming, J.E.* (1992). Stress resistance of *Drosophila* transgenic for bovine CuZn superoxide dismutase. *Free Rad. Res. Comm.* **17,** 73-85.

Reveillaud, I., Neidzweicki, A., Bensch, K.G. and *Fleming, J.E.* (1991). Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. *Mol. Cell. Biol.* **11,** 632-640.

Ribeiro, J., Hazzard, J., Nussenzveig, R.H., Champagne, D.E. and *Walker, F.A.* (1993). Reversible binding of nitric oxide by a salivary heme protein from a bloodsucking insect. *Science* Wash. DC **260,** 539-541.

Ribeiro, J.M.C. and *Nussenzveig, R.H.* (1993). Nitric oxide synthase activity from a hematophagous insect salivary gland. *FEBS Lett.* **330,** 165-168.

Robinson, J.R. and *Beatson, E.P.* (1985). Enhancement of dye-sensitized phototoxicity to housefly larvae *in vivo* by dietary ascorbate siazobicyclooctane and other additives. *Pestic. Biochem. Physiol.* **24,** 375-383.

Rose M.R., Graves, J.L. and *Hutchinson, E.W.* (1990). The use of selection to probe patterns of pleiotropy in fitness characters. In: *Insect Life Cycles: Genetics, Evolution nd Coordination,* 29-42, (Ed. E.Gilbert), London, Springer.

Service, P.M. (1987). Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **60,** 321-326.

Service, P.M., Hutchinson, E.W., MacKinley M.D. and *Rose, M.R.* (1985). Resistance to environmental stress in *Drosophila melanogaster* selected for postponed sene-scence. *Physiol. Zool.* **58,** 380-389.

Šešlija, D., Blagojević, D., Spasić, M. and *Tucić, N.* (1999). Activity of superoxide dismutase and catalase in the bean weevil (*Acanthoscelides obtectus*) selected for postponed senescence. *Exp. Gerontol.* **34,** 185-195.

Seto, N.O.L., Hayashi, S. and *Tener, G.M.* (1990). Overexpression of Cu-Zn superoxide dismutase does not affect life-span. *Proc. Natl. Acad. Sci. USA* **87,** 4270-4274.

Smith, J. and *Shrift, A.* (1979). Phylogenetic distribution of glutathione peroxidase. *Comp. Biochem. Physiol.* **63B,** 39-44.

Sohal, R.S. (1985). Aging in insects. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* 10, 595-631, (Eds. G.A. Kerkut and L.I. Gilbert), New York, Pergamon Press.

Sohal, R.S. (1988). Effect of hydrogen peroxide administration on life span, superoxide dismutase, catalase, and glutathione in the adult housefly, *Musca domestica. Exp. Gerontol.* **23,** 211-216.

Sohal, R.S., Agarwal, A., Agarwal, S. and *Orr, W.C.* (1995). Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age-related oxidative damage and increases metabolic potential in *Drosophila melanogaster. J. Biol. Chem.* 270, 15671-15674.

Sohal, R.S. and *Allen, R.G.* (1986). Relationship between oxygen metabolism, aging and development. *Adv. Free Rad. Biol. Med.* **2,**117-160.

Sohal, R.S. and Allen, R.G. (1990). Oxidative stress as a causal factor in differentiation and aging: A unifying hypothesis. *Exp. Gerontol.* **25,** 499-522.

Sohal, R.S., Allen, R.G., Farmer, R.K., Newton, R.K. and *Toy, P.L.* (1985). Effects of exogenous antioxidants on the level of endogenous antioxidants, lipid-soluble flourescent material and life-span in the housefly, *Musca domestica. Mech. Ageing Dev.* **31,** 329-336.

Sohal, R.S., Allen, R.G. and *Nations, C.* (1986). Oxygen free radicals play a role in cellular differentiation: A hypo-thesis. *J. Free Rad. Biol. Med.* **2,** 175-181.

Sohal, R.S., Arnold, L. and *Orr, W.C.* (1990). Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/$NADP^+$ and NADH/NAD^+ in *Drosophila melanogaster. Mech. Ageing Dev.* **56,** 223-235.

Sohal, R.S., Farmer, K.J., Allen, R.G. and *Ragland, S.S.* (1984). Effects of diethyldithiocarbamate on life span, metabolic rate, superoxide dismutase, inorganic peroxides and glutathione activity in the adult male housefly, *Musca domestica. Mech. Ageing Dev.* **24,** 175-183.

Summers, C.B. and *Felton, G.W.* (1993). Antioxidant role of dehydroascorbic acid reductase in insects. *Biochim. Biophys. Acta,* **1156,** 235-238.

Toru, A. (1994). Superoxide generation *in vitro* in lepidopteran larval hemolymph. *J. Insect Physiol.* **40.** 165-171.

Wadano, A., Tanaka, Y. and *Matumoto, M.* (1993). 3-Hydroxykynurenine as a radical scavenger in the blowfly, *Aldrichina grahami. Insect Biochem. Mol. Biol.* 23, 919-925.

Whitten, M.M.A. and *Ratcliffe, N.A.* (1999). *In vitro* superoxide dismutase activity in the haemolymph of the West Indian leaf cockroach, *Blaberus discoidalis. J. Insect Phy-siol.* **45,** 667-675.

Wijesundara, M.B.J. and *Berger, S.* (1994). The redox pair vitamin E and vitamin C, a ^{13}C-NMR study. *Liebigs Ann. Chem.* 1239-1241.

Worland, R., Block, W. and *Grubor-Laji, G.* (2000). Survival of *Heleomyza borealis* (Diptera, Heleomyzidae) larvae down to - 60 C. *Physiol. Entomol.* 25, 1-5.

Worland, R., Grubor-Laji, G. and *Monticl, P.O.* (1998). Partial desiccation induced by sub-zero temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus arcticus* (Tullberg). *J. Insect Physiol.* **44,** 211-219.

Zwaan, B.J., Bijlsma, R. and *Hoekstra, R.F.* (1991). On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to preadult breeding conditions. *Heredity* **66,** 29-39.