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Original article

Antioxidant enzymes in the honey bee, *Apis mellifera*[1]

Gunter F. WEIRICH, Anita M. COLLINS*, VIRGINIA P. WILLIAMS

Bee Research Laboratory, ARS, USDA, Bldg. 476, BARC-East,
10300 Baltimore Ave., Beltsville, MD 20705-2350, USA

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Abstract – Catalase (CAT), glutathione *S*-transferase (GST) and superoxide dismutase (SOD) activities were determined in postmitochondrial fractions of tissue homogenates (spermathecae, muscle and ventriculi), in hemolymph plasma, and in semen of honey bees. The highest CAT activity was found in semen (4.8 mU/μg fresh weight), and the enzyme was confined to the spermatozoa. CAT and GST activities of ventriculi exceeded those of other tissues and hemolymph, CAT being highest in mated queen ventriculi (2.7 mU/μg) and GST highest in worker ventriculi (10 mU/mg). Spermathecae of mated queens had higher CAT and GST activities (0.84 mU/μg, and 2.4 mU/mg, respectively) than virgin spermathecae (0.15 mU/μg, and 1.6 mU/mg). SOD activities (15–59 mU/μg) varied less than activities of CAT or GST between tissues. Seminal plasma contained two thirds of the total SOD activity of semen and one third was in the spermatozoa. The substantial activities of all three enzymes in spermathecae of mated queens suggest their involvement in the long-term protection of the spermatozoa from oxidative stress.

***Apis mellifera* / catalase / glutathione *S*-transferase / superoxide dismutase**

1. INTRODUCTION

All aerobic organisms generate reactive oxygen species in the process of their oxidative metabolism [for reviews, see Felton, 1995; Felton and Summers, 1995; Michiels et al., 1994; Pardini, 1995]. These reactive oxygen species include the superoxide anion, $O_2^{\bullet -}$, the hydroperoxyl radical, $HO_2^{\bullet}$, hydrogen peroxide, H_2O_2, and the hydroxyl radical, $^{\bullet}OH$, all intermediates in the reduction of O_2 to H_2O and derived from enzymatic reactions and autooxidation of redox-active chemicals occurring in living cells. Animals also take up prooxidant

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* Correspondence and reprints
E-mail: CollinsA@ba.ars.usda.gov

allelochemicals with their diet, and these oxidants give rise to reactive oxygen species. The reactive oxygen species can cause oxidation of proteins, RNAs, and DNAs, and peroxidation of membrane lipids. These destructive reactions contribute to the processes of aging, carcinogenesis and cell death. To protect against the effects of "oxidative stress", organisms have a variety of detoxifying enzymes at their disposal, such as superoxide dismutase, catalase, glutathione *S*-transferase, glutathione peroxidase and glutathione reductase, all of which have been reported to occur in insects (Ahmad et al., 1991; Felton and Summers, 1995; Joanisse and Storey, 1996). SODs convert $O_2^{\bullet -}$ to H_2O_2 and O_2 (McCord and Fridovich, 1969). H_2O_2 is eliminated by the action of CATs (Aebi, 1984) and glutathione peroxidases (Mannervik, 1985). GSTs are involved in the detoxification of a wide variety of toxic compounds by conjugating them to glutathione and thus facilitating their removal from the organism (Hinton, et al. 1995; Jakoby, 1985; Lee, 1991). Some GSTs also have glutathione peroxidase activity, thus providing an oxygen-detoxifying function [i.e., reduction of lipid peroxides (Mannervik, 1985)]. This activity is particularly important in invertebrates because they are deficient in the other (vertebrate-type, selenium-dependent) glutathione peroxidases (Ahmad et al., 1991; Pardini, 1995). In reactions catalyzed by glutathione peroxidase, glutathione (GSH) is oxidized to GSSG, which in turn is reduced by glutathione reductases. The activity of these enzymes is correlated with the function and metabolic activity (potential oxidative stress) of tissues, and it is usually high in herbivorous species feeding on plants containing reactive-oxygen-species generating chemicals (Aucoin et al., 1991; Pardini, 1995).

In the spermatheca of the queen honey bee (*Apis mellifera* L.), spermatozoa are kept alive and capable of fertilization for several years (Koeniger, 1986). The spermatheca is a fluid-filled sphere formed by a thin chitin membrane surrounded by a single layer of columnar epithelial cells and a dense tracheal net (Poole, 1970; Ruttner et al., 1971). This organ provides a specialized physiological environment, in which the spermatozoa are densely packed and show decreased metabolism. For the prolonged survival of these non-dividing and non-regenerating cells, protection from oxidative stress should be of paramount importance.

Antioxidant enzymes have been implicated in the protection of mammalian spermatozoa against oxidative damage [for review, see Hinton et al., 1995]. The motility of bull spermatozoa after freezing and thawing was found to be prolonged by antioxidants (Lindemann et al., 1988), and Donoghue & Donoghue (1997) have reported improvements in survival, membrane integrity and motility of turkey spermatozoa by addition of antioxidants to the storage medium.

To determine the possible implication of antioxidant enzymes in the long-term survival of honey bee spermatozoa in the spermatheca, we measured the activities of three of these enzymes, SOD, CAT and GST, in the 16 000 *g* supernatant of homogenized spermathecae (containing spermathecal fluid, cytosol and microsomes), and for comparison, in the 16 000 *g* supernatants of homogenized muscle and ventriculi (the major part of the intestinal tract), and in hemolymph plasma of queens (virgin and mated) and worker bees. As a related study on semen storage had shown that honey bee semen survives for many months even at room temperature (Collins, 2000), we also assayed a sample of semen for the presence of SOD, CAT and GST. This paper represents the first report on antioxidant enzymes in insect semen and on the tissue distribution of these enzymes in bees.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Bees

Queens, mated and virgin, were purchased from one bee breeder, Wilbanks Apiaries, Claxton, GA, to ensure genetic similarity of the samples, and workers (raised in our apiary) were of the same stock. Queens were either held in a queen bank (queenless colony of bees, maintained to care for groups of caged queens), or introduced into small, five-frame nucleus colonies and allowed to lay eggs. Colonies (queen banks and nuclei) were fed sugar syrup if there was no natural nectar flow, and pollen supplement, so that the queens were well fed. Virgin queens were sacrificed at the age of three weeks, and mated queens, two to three months after mating.

2.3. Enzyme preparations

Hemolymph and tissues were collected from two samples of worker bees (seven per sample), three samples of virgin queens (seven per sample) and two samples of mated queens (six to seven per sample). Hemolymph was collected in glass capillary tubes from a small dorsal opening made in the second abdominal segment and mixed with an equal volume of ice-cooled 100 mM potassium phosphate buffer, pH 7.0, containing 0.01% phenylthiourea (PPT buffer). To remove hemocytes the diluted hemolymph was centrifuged for 10 min at 16 000 *g* and 5 ºC and the supernatant (plasma) was saved for enzyme assays.

Spermathecae, thoracic muscle and ventriculi were dissected, freed from extraneous tissues (and ventriculi contents), and rinsed in ice-cooled PPT buffer. For the wet weight determinations the buffer was removed by low speed centrifugation (325 *g*, 5 ºC), aspiration, and insertion of capillary tubes into the pelleted tissues. The tissues were homogenized by hand with five volumes of PPT buffer in glass micro homogenizers. The homogenates were centrifuged for 20 min at 16 000 *g* (5 ºC), eliminating cell debris, nuclei, mitochondria and probably part of the peroxisomes (Geller and Winge, 1984; Mertens-Strijthagen et al., 1979; Weirich and Adams, 1984); and the supernatants, containing the postmitochondrial fractions of the tissues (and spermathecal fluid), were saved for enzyme assays.

Semen was collected by the standard method. Sexually mature drones were stimulated by pressing on the ventral thorax. This induced partial or complete eversion of the penis. If only partial eversion occurred, further pressure was applied to the abdomen to complete the eversion. After eversion, the semen and accompanying mucus plug were exposed. Using a dissecting microscope, the semen (< 1µl/drone) without the mucus was drawn into a Harbo syringe [a Gilmont micrometer syringe, connected by fluid-filled tubing to a glass tip and capillary collection tube (Harbo, 1985)]. Thirty-five microliters of semen were collected from a total of about 50 drones, transferred into a microcentrifuge tube, and mixed with an equal volume of ice-cooled potassium phosphate buffer (100 mM, pH 7).

Aliquots of diluted semen and of the supernatants of hemolymph and tissue homogenates were stored at –85 ºC until used for the enzyme assays. Some aliquots of the diluted semen were centrifuged for 20 min at 16 000 *g* (5 ºC) after thawing, and the supernatants (diluted seminal plasma) and resuspended pellets (spermatozoa) were assayed separately.

2.4. Enzyme assays

The progress of all enzyme reactions was monitored at 25 ºC in a Model DU640 spectrophotometer (Beckman, Fullerton, CA). Assay volumes were 200 µl. The assay procedures were validated by experiments involving bovine liver CAT, bovine liver GST, and bovine erythrocyte SOD. Conversion of 1.0 µmole/min was used as unit definition for CAT and GST, and activities are shown as milliunits (mU) per fresh-weight equivalent (mg or µg). For the definition of SOD activity units see Section 2.4.3.

2.4.1. Catalase

CAT activities were determined by the method of Aebi (1984). Assay mixtures contained 10 mM H_2O_2, and varying amounts of enzyme preparation in 50 mM potassium phosphate buffer, pH 7.0. Enzyme activities were calculated using 0.0394 $mM^{-1} \cdot cm^{-1}$ as absorption coefficient at 240 nm. Ninety-five to 100 percent of the activities in all but the muscle preparations were destroyed by boiling. In muscle only 70–75% of the apparent catalase activity was eliminated.

2.4.2 Glutathione S-transferase

GST activities toward 1-chloro-2,4-dinitrobenzene were determined as described by Warholm et al. (1985) except the glutathione concentration was increased to 2.5 mM. *S*-(2,4-dinitrophenyl)glutathione formation was measured at 340 nm ($\varepsilon = 9.6\ mM^{-1} \cdot cm^{-1}$). To calculate the enzyme activities, the rates were corrected for the nonenzymatic component of that reaction determined in parallel incubations without enzyme. Enzyme preparations from the ventriculus (although kept in ice) showed a decrease in GST activity when assayed repeatedly over the course of a day. As reported by Clark et al. (1985), loss of GST activity from homogenates of the New Zealand grass grub, *Costelytra zealandica*, was prevented by addition of 5 mM glutathione. We found addition of 5 mM glutathione to be effective in minimizing GST loss in ventriculus enzyme preparations as well and accordingly, it was added to all bee ventriculus samples included in this study. (In the assay mixture the glutathione concentration was reduced to 2.5 mM.) Boiling eliminated 94–100% of the GST activities.

2.4.3. Superoxide dismutase

SOD activities were determined according to Paoletti et al. (1986) and Paoletti and Mocali (1990) with slight modifications. This method is 10 to 40 times as sensitive as other commonly used assay procedures. The assay mixtures (200 µl) consisted of 75 mM triethanolamine, 75 mM diethanolamine, pH 7.5; 1.25 mM manganese chloride, 2.5 mM EDTA, 0.3 mM NADH, 1.0 mM mercaptoethanol, and varying amounts of enzyme preparation. The unit of activity is defined as the amount of enzyme causing 50 percent inhibition of the rate of the superoxide-driven NADH oxidation (McCord and Fridovich, 1969). Accordingly, each determination consisted of three assays run at different enzyme concentrations, and the amount of enzyme preparation (µg fresh-weight equivalents) required for 50% inhibition was computed by semilog linear regression (Paoletti et al., 1986; Paoletti and Mocali, 1990). Boiled enzyme preparations caused a marginal inhibition of NADH oxidation (<10%) in the upper range of the enzyme concentrations, possibly resulting in a slight overestimation of some of the enzyme activities. The cause of this activity is not known. It may reflect a non-enzymatic dismutation of the superoxide radical facilitated by some component(s) of the enzyme preparations.

2.5. Statistical analysis

The three variables CAT, GST and SOD were analyzed as two-factor (caste/mating

state; tissue) general linear models analyses of variance using PROC MIXED (SAS Institute, 1997) in a split plot. One part (I) included all three castes or mating states with all tissues but spermatheca, the second (II) included only queens (mated, virgin) and all tissues including spermatheca. Averages of the measurements on multiple aliquots of each pooled sample were used as the data set for the analysis. All variables showed variance heterogeneity, so CAT and GST were natural-log transformed, ln(x), and the variance grouping technique was used with the three variables. Sidak means comparison was used for the appropriate pair-wise mean comparisons. For each variable, the significance level, $\alpha = 0.05$, is the experiment value. Semen was not included in the statistical analysis, as only one sample was assayed for enzyme activity.

3. RESULTS

Enzyme activities of some tissues showed substantial differences between bee samples of the same caste and/or mating state. These differences are reflected in the standard deviations shown in Figures 1–3. Although these differences could not be attributed to any particular cause, they suggest that the enzyme activities

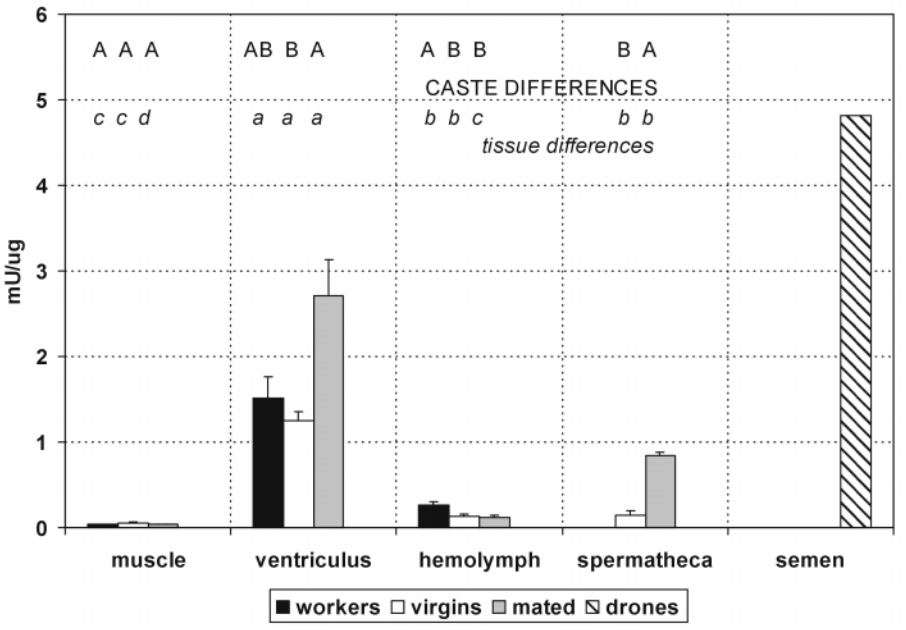

Figure 1. CAT activities in 16 000 *g* supernatants of homogenized muscle, ventriculi and spermathecae, and in hemolymph plasma of workers, virgins and mated queens; and in semen. Activities were determined for two composite samples obtained from workers, three from virgin queens, two from mated queens, and one semen sample, prepared as described in Materials and methods. Two or more aliquots of each sample were analyzed, and data are shown as means ± STD. Further statistical analysis was done as described in Materials and methods: Caste (or mating state) means within tissue/hemolymph with different (capital) letters are statistically different at the $P \leq 0.05$ significance level. Tissue/hemolymph means within caste/mating state (workers, virgins, mated queens) with different (lower case) letters are statistically different at the $P \leq 0.05$ significance level.

can be affected by external or internal factors that differed between different samples of workers, virgins and mated queens. Nevertheless certain patterns were obvious from comparisons of average data for each enzyme, tissue, and caste or mating state.

The interaction term (caste/mating state by tissue) from the statistical analysis was significant for all enzymes (F = 9.95, 14.38, 10.97; df = 4; P = 0.002, 0.0203, 0.0023). This simply means that the relative levels of enzyme activity were not the same for all within-caste/mating state or within-tissue comparisons.

3.1. Catalase

CAT activities were highest in ventriculi (Fig. 1; I. F = 587.9, df = 2, $P < 0.0001$; II. F = 269.96, df = 3, $P < 0.0001$) and in semen. The activity of semen was confined to the spermatozoa. After 16 000 *g* centrifugation of the previously frozen semen nearly all activity was found in the pellet and less than one percent in the supernatant (diluted seminal plasma). The activities in ventriculi of mated queens were higher than those in ventriculi of workers or virgin queens. However, only virgins and mated queens differed at the $P \leq 0.05$ significance level (F = 25.25, df = 1, P = 0.0009). Spermathecae of mated queens showed five times higher activities than those of virgins. CAT activities in hemolymph were generally low, somewhat higher in workers than virgin or mated queens. Muscle activities were marginal in all three groups.

3.2. Glutathione *S*-transferase

The highest GST activities were found in the ventriculi (Fig. 2; I. F = 381.44,

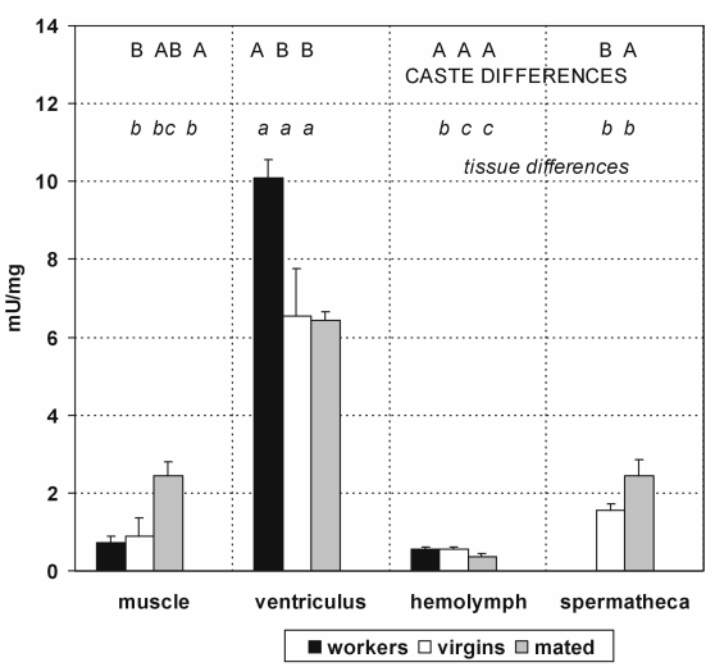

Figure 2. GST activities in 16 000 *g* supernatants of homogenized muscle, ventriculi and spermathecae, and in hemolymph plasma of workers, virgins and mated queens. Details as in Figure 1.

df = 2, P = 0.0003; II. F = 179.54, df = 3, P = 0.0001), and ventriculi of workers were about 50 percent more active than those of virgins or mated queens. Muscles of mated queens had higher activity than worker muscles, and virgin muscles were not significantly different from either. The activity in spermathecae of mated queens was higher than in virgin spermathecae. Hemolymph activities were low in all three groups. Assays of complete semen, due to high intrinsic 340 nm absorbance and relatively low GST activity, did not yield reproducible results. No trace of activity was found in the 16 000 *g* supernatant of semen (diluted seminal plasma).

3.3. Superoxide dismutase

SOD activities (Fig. 3) did not show the large differences between tissues seen with the other two enzymes, but some differences were significant (I. F = 11.28, df = 2, P = 0.005; II. F = 22.27, df = 3, P = 0.0004). Muscle preparations of mated queens had about twice the activity found in virgin or worker muscle. Activities in ventriculi, hemolymph and spermathecae did not differ significantly between workers, virgins and mated queens. Activity in semen was comparable to that of mated-queen spermathecae. Unlike CAT and GST, the SOD in semen was not entirely cell-bound. After a 20-min 16 000 *g* centrifugation of

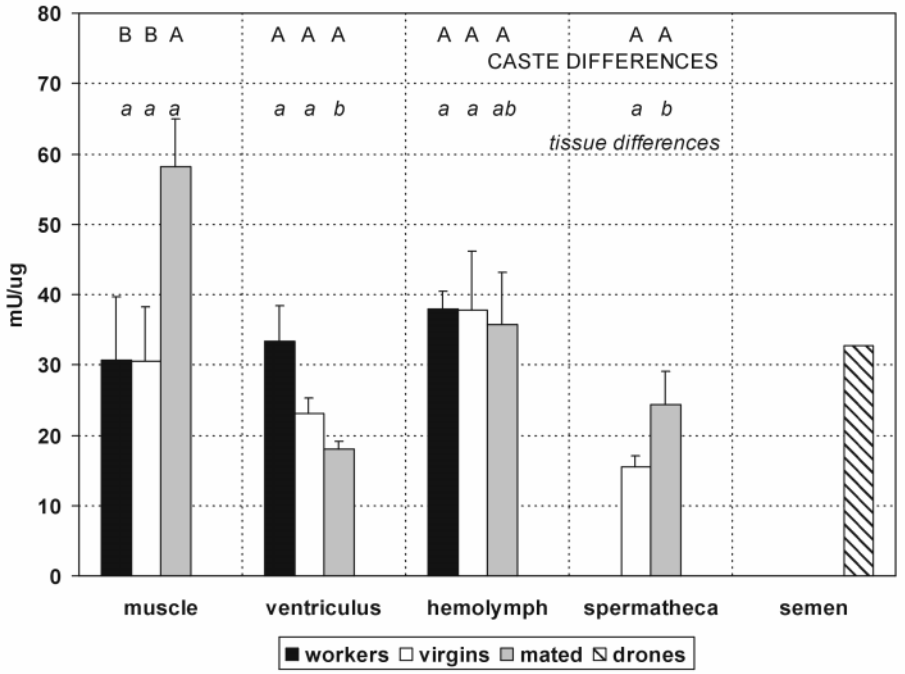

Figure 3. SOD activities in 16 000 *g* supernatants of homogenized muscle, ventriculi and spermathecae, and in hemolymph plasma of workers, virgins and mated queens; and in semen. Details as in Figure 1.

the previously frozen preparation, 69 percent of the activity was recovered in the supernatant and 31 percent in the pellet, indicating that a large part of the total SOD was contained in the seminal plasma.

4. DISCUSSION

The enzyme activities shown in Figures 1–3 represent the combined activities of microsomes and cytosol for muscle and ventriculi; of microsomes, cytosol and spermathecal fluid for spermathecae; and the complete activities of semen (spermatozoa and seminal plasma).

CAT is generally believed to be primarily located in peroxisomes (Tolbert, 1978). Thus, CAT may have been lost during the centrifugation of the tissue homogenates. However, soluble CAT has been found in mammalian (Masters et al., 1986) as well as insect species (Aucoin et al., 1991; Joanisse and Storey, 1996), and larval homogenates of lepidopteran species have been shown to contain substantial CAT activities in mitochondrial, microsomal and cytosol fractions (Ahmad et al., 1988a, 1988b, 1990). The midgut of honey bee workers contains CAT in peroxisomal as well as soluble fractions (Jimenez and Gilliam, 1996).

In our studies the highest CAT activities were recorded in semen. This activity was cell-bound and sedimented during 16 000 *g* centrifugation of the previously frozen diluted semen. It could provide protection against H_2O_2 generated internally or entering the cells by diffusion. The high activity of CAT in the ventriculi suggested a high dietary exposure to H_2O_2 or H_2O_2-generating chemicals, higher in the mated queens in accordance with their higher food intake (Allen, 1960). In this context, it is interesting to note that honey contains H_2O_2, produced by glucose oxidase, an enzyme present in honey (White et al., 1963).

Of particular interest in relation to sperm preservation is the markedly enhanced CAT activity in spermathecae of mated as compared to virgin queens. Unlike the CAT activity of semen, the activity of spermathecae was not sedimented at 16 000 *g* and thus probably did not derive from semen. The increased activity in the spermathecae of mated queens appears to be generated by the spermatheca/spermathecal gland complex, presumably to provide protection of the spermatozoa from external H_2O_2.

Muscle preparations showed only marginal CAT activities, which even at these low levels were probably somewhat overestimated due to the nonenzymatic (heat-stable) component of the H_2O_2 decomposition. It should be noted, however, that the enzyme preparations consisted only of the postmitochondrial fractions from which, depending on their size, most or all of the peroxisomes may have been excluded. Perhaps due to the gentle homogenization (by hand) there was no significant release of the enzyme from the peroxisomes. Furthermore, our results suggest that bee muscle does not contain soluble or microsomal CAT.

GSTs have been found in cytosol, mitochondria and microsomes (Mannervik, 1985). As with CAT, the highest GST activities among the bee tissues were found in the ventriculi, but in the case of GST there was no difference between virgins and mated queens, and the workers had higher activities than the queens.

All eukaryotes are thought to have a cytosolic and a mitochondrial form of SOD, in mammals characterized by copper and zinc or manganese content, respectively (Geller and Winge, 1984). Evidence for the dual localization and the existence of SODs with different metal content has also been reported for several lepidopteran species (Ahmad et al., 1988a, 1988b, 1990, 1991).

Our data for bee muscle, ventriculi and spermathecae reflect the activities of the soluble SODs. The similarity between the activities of the three tissues and the high

activities in hemolymph plasma were unexpected. It is possible that the mitochondria (not included in our enzyme preparations) contain a major portion of the total SOD activities in bees and their inclusion would yield a different tissue profile. SOD was the only enzyme found in both supernatant and pellet after the centrifugation of semen. Thus, SOD is present not only inside the spermatozoa (mitochondria and/or cytosol), but also in the seminal plasma.

Tissue distribution of antioxidant enzymes in insects has received very little attention. In most studies homogenates of whole insects were used to obtain the enzyme preparations. Ahmad et al. (1991) analyzed the distribution of six enzymes in sonicated 850 *g* supernatants of tissue homogenates and in hemolymph of *Trichoplusia ni* larvae. These authors found substantial CAT, GST and SOD activities in various segments of the gut and in muscle, and marginal or no activity in hemolymph. The hemolymph SOD was confined to the hemocytes, and only a trace of activity was found in the plasma. Compared to *T. ni*, the most noticeable differences in the enzyme profiles of honey bees were the low CAT and GST activities in muscle and the high SOD activities in hemolymph plasma. The low CAT and GST activities may be related to the absence of mitochondria and peroxisomes from the bee enzyme preparations and the fact that, unlike Ahmad et al. (1991), we did not sonicate the homogenates to release enzymes confined to these organelles.

The activities of CAT and GST were higher in the spermathecae of mated queens than in those of virgins. Seminal plasma, containing neither soluble CAT nor soluble GST, could not have contributed to these increased activities. Some enzymes could have been released by the spermatozoa into the spermathecal fluid, but in view of the very low metabolic activity of the stored spermatozoa, it is unlikely that they would have a significant role in controlling or maintaining the composition of the spermathecal fluid. In semen kept for five months at 23 ^{o}C none of the CAT was released from the spermatozoa (Weirich, Collins and Williams, unpublished observation). Therefore, the spermatheca or spermathecal gland appear to be the more plausible sources of increased or sustained enzyme activities.

CAT and GST activities in spermathecae of mated queens were exceeded only by the activities in their ventriculi (and in the case of CAT, by semen). SOD activities of mated-queen spermathecae were in the same range as those of ventriculi and hemolymph. As an organ not involved in any major metabolic activity, the spermatheca of the mated queen showed remarkably high activities for all three enzymes in the postmitochondrial fraction (spermathecal fluid, microsomes and cytosol) of the homogenized tissue. It is therefore reasonable to conclude that these spermathecal enzymes contribute to the protection of the spermatozoa from oxidative stress and thereby help to facilitate their long-term survival. Based on our results it would be interesting to test whether the addition of CAT, GST, SOD or other antioxidant enzymes to semen stored in vitro could further extend the survival of the spermatozoa and improve the success in the preservation and propagation of honey bee germplasm.

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Résumé – Enzymes antioxydants chez l'abeille, *Apis mellifera*. Dans la spermathèque de la reine d'abeilles, les

spermatozoïdes restent vivants et leur capacité de fécondation se maintient durant des années. On sait que des enzymes antioxydants sont impliqués dans la protection des spermatozoïdes des mammifères contre les dégâts dus à l'oxydation. Afin de déterminer le rôle éventuel des enzymes antioxydants dans la survie à long terme des spermatozoïdes de l'abeille, nous avons mesuré les activités de trois de ces enzymes, la catalase (CAT), la glutathione *S*-transférase (GST) et la superoxyde dismutase (SOD), dans le sperme, la spermathèque (surnageant de l'homogénat après centrifugation à 16 000 *g*, ce qui correspond à la fraction post-mitochondriale) et, pour comparaison, dans le muscle, les ventricules (surnageant de l'homogénat après centrifugation à 16 000 *g*) et le plasma de l'hémolymphe. Les échantillons de tissus et d'hémolymphe ont été prélevés sur des reines vierges, des reines fécondes et des ouvrières (2–3 échantillons de 6–7 abeilles chacun). Un échantillon composite de sperme a été récolté à partir d'environ 50 mâles. L'activité CAT la plus élevée (Fig. 1) a été trouvée dans le sperme (4,8 mU/µg de poids frais) où l'enzyme est limitée aux spermatozoïdes. Les activités de la CAT et de la GST (Figs. 1 et 2) des ventricules ont dépassé celles des autres tissus et de l'hémolymphe, la CAT étant la plus active dans les ventricules des reines fécondes (2,7 mU/µg) et la GST la plus active dans les ventricules d'ouvrières (10 mU/mg). Les spermathèques des reines fécondes ont montré une activité de la CAT cinq fois plus élevée (0,84 mU/µg) que les spermathèques des reines vierges. L'activité de la GST dans les spermathèques des reines fécondes (2,4 mU/mg) était également plus élevée que dans les spermathèques des reines vierges. En raison de l'absorbance propre élevée à 340 nm la GST n'a pas pu être déterminée dans le sperme. L'activité de la SOD des divers tissus (Fig. 3) a moins varié (15–59 mU/µg) que celles de la CAT ou de la GST. Les deux tiers de l'activité totale de la SOD dans le sperme, provenait du plasma séminal et un tiers des spermatosoïdes. On conclut d'après les activités importantes des trois enzymes dans la spermathèque des reines fécondes que ces enzymes aident la survie à long terme des spermatozoïdes en les protégeant des dégâts oxydatifs.

***Apis mellifera* / spermathèque / catalase / glutathion *S*-transférase / superoxyde dismutase**

Zusammenfassung – Antioxidierende Enzyme bei der Honigbiene, *Apis mellifera.* In der Spermatheka der Königinnen der Honigbienen werden Spermatozoen lebend gespeichert und bleiben für mehrere Jahre befruchtungsfähig. Der Schutz der Spermatozoen von Säugetieren gegen eine oxidative Schädigung soll durch antioxidierende Enzyme erfolgen. Um eine mögliche Rolle von antioxidierenden Enzymen auf eine Langzeit-Speicherung der Spermatozoen zu untersuchen, bestimmten wir die Aktivitäten von 3 Antioxidasen: Katalase, (CAT), Glutathion *S*-Transferase (GST) und Superoxid Dismutase (SOD). Außer im Sperma wurden die Aktivitäten in 4 weiteren Organen untersucht, in der Spermatheka (Überstand des Homogenats nach Zentrifugation bei 16 000 *g*, das entspricht der postmitochondrialen Fraktion), und zum Vergleich in Muskeln, Ventrikeln (bei beiden der 16 000 *g* Überstand des Homogenats) und im Plasma der Hämolymphe. Gewebe- und Hämolymphproben wurden von unbegatteten, von begatteten Königinnen und von Arbeiterinnen gesammelt (2–3 Proben von jeweils 6–7 Bienen). Eine Sammelprobe von Sperma wurde von ~50 Drohnen gewonnen. Die höchste CAT Aktivität (Abb. 1) wurde im Sperma nachgewiesen (4.8 mU/µg Frischgewicht) wobei das Enzym an die Spermatozoen gebunden war. CAT und GST Aktivitäten (Abb. 1 und 2) der Ventrikel übertrafen die der anderen Gewebe. Die CAT Aktivität war am höchsten in den Ventrikeln der begatteten Königinnen

(2.7 mU/μg) und GST am höchsten in den Ventrikeln der Arbeiterinnen (10 mU/mg). Spermatheken mit Spermatozoen der begatteten Königinnen zeigten eine fünfmal höhere CAT Aktivität (0.84 mU/μg) als leere Spermatheken von unbegatteten. GST Aktivitäten in Spermatheken begatteter Königinnen (2.4 mU/mg) waren ebenfalls höher als die der unbegatteten Königinnen. Auf Grund der hohen spermaspezifischen Absorption bei 340 nm konnte GST im Sperma nicht bestimmt werden. Bei den SOD Aktivitäten (Abb. 3) der Gewebe gab es geringere Unterschiede (15–59 mU/μg) als bei den Aktivitäten von CAT oder GST. Die Spermaflüssigkeit enthielt 2/3 der Gesamtaktivität von SOD, 1/3 war an die Spermatozoen gebunden. Aus den substantiellen Aktivitäten aller 3 Enzyme in Spermatheken von begatteten Königinnen wurde geschlossen, dass diese Enzyme ein langzeitiges Überleben der Spermatozoen durch einen Schutz vor oxidativen Schäden unterstützen.

***Apis mellifera* / Spermatheka / Katalase / Glutathion *S*-Transferase / Superoxid Dismutase /**

REFERENCES

Aebi H. (1984) Catalase in vitro, Methods Enzymol. 105, 121–126.

Ahmad S., Pritsos C.A., Bowen S.M., Heisler C.R., Bloomquist G.J., Pardini R.S. (1988a) 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. Commun. 4, 403–408.

Ahmad S., Pritsos C.A., Bowen S.M., Heisler C.R., Bloomquist G.J., Pardini R.S. (1988b) Subcellular distribution and activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the Southern armyworm, *Spodoptera eridania,* Arch. Insect Biochem. Physiol. 7, 173–186.

Ahmad S., Pritsos C.A., Pardini R.S. (1990) Antioxidant enzyme activities in subcellular fractions of larvae of the black swallowtail butterfly, *Papilio polyxenes*, Arch. Insect Biochem. Physiol. 15, 101–109.

Ahmad S., Duval D.L., Weinhold L.C., Pardini R.S. (1991) Cabbage looper antioxidant enzymes: Tissue specificity, Insect Biochem. 21, 563–572.

Allen M.D. (1960) The honey bee queen and attendants, Anim. Behav. 8, 201–208.

Aucoin R.R., Philogène B.J.R., Arnason J.T. (1991) Antioxidant enzymes as biochemical defenses against phototoxin-induced oxidative stress in three species of herbivorous Lepidoptera, Arch. Insect Biochem. Physiol. 16, 139–152.

Clark A.G., Dick G.L., Martindale S.M., Smith J.N. (1985) Glutathione *S*-transferases from the New Zealand grass grub, *Costelytra zealandica*, Insect Biochem. 15, 35–44.

Collins A.M. (2000) Survival of honey bee (Hymenoptera: Apidae) spermatozoa stored at above-freezing temperatures, J. Econ. Entomol. 93, 568–571.

Donoghue A.M., Donoghue D.J. (1997) Effects of water- and lipid-soluble antioxidants on turkey sperm viability, membrane integrity and motility during liquid storage, Poultry Sci. 76, 1440–1445.

Felton G.W. (1995) Antioxidant defenses of invertebrates and vertebrates, in: Ahmad S. (Ed.), Oxidative Stress and Antioxidant Defenses in Biology, Chapman & Hall, New York, pp. 356–434.

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

Geller B.L., Winge D.R. (1984) Subcellular distribution of superoxide dismutase in rat liver, Methods Enzymol. 105, 105–114.

Harbo J.R. (1985) Instrumental insemination of queen bees – Parts 1 & 2, Am. Bee J. 125, 197–202; 282–287.

Hinton B.T., Palladino M.A., Rudolph D., Labus J.C. (1995) The epididymis as protector of maturing spermatozoa, Reprod. Fertil. Dev. 7, 731–745.

Jakoby W.B. (1985) Glutathione transferases: An overview, Methods Enzymol. 113, 495–499.

Jimenez D.R., Gilliam M. (1996) Peroxisomal enzymes in the honey bee midgut, Arch. Insect Biochem. Physiol. 31, 87–103.

Joanisse D.R., Storey K.B. (1996) Oxidative stress and antioxidants in overwintering larvae of cold-hardy goldenrod gall insects, J. Exp. Biol. 199, 1483–1491.

Koeniger G. (1986) Reproduction and mating behavior, in: Rinderer T.E. (Ed.), Bee Genetics and Breeding, Academic Press, New York, pp. 255–280.

Lee K. (1991) Glutathione *S*-transferase activities in phytophagous insects: Induction and inhibition by plant phototoxins and phenols, Insect Biochem. 21, 353–361.

Lindemann C.B., O'Brien J.A., Giblin F.J. (1988) An investigation of the effectiveness of certain antioxidants in preserving the motility of reactivated bull sperm models, Biol. Reprod. 38, 114–120.

Mannervik B. (1985) Glutathione peroxidase, Methods Enzymol. 113, 490–495.

Masters C., Pegg M., Crane D. (1986) On the multiplicity of the enzyme catalase in mammalian liver, Mol. Cell. Biochem. 70, 113–120.

McCord J.M., Fridovich I. (1969) Superoxide dismutase, J. Biol. Chem. 244, 6049–6055.

Mertens-Strijthagen J., De Schrijver C., Wattiaux-De Coninck S., Wattiaux R. (1979) A centrifugation study of rat-liver mitochondria, lysosomes and peroxisomes during the perinatal period, Eur. J. Biochem. 98, 339–352.

Michiels C., Raes M., Toussaint O., Remacle J. (1994) Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress, Free Rad. Biol. Med. 17, 235–248.

Paoletti F., Mocali A. (1990) Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation, Methods Enzymol. 186, 209–220.

Paoletti F., Aldinucci D., Mocali A., Caparrini A. (1986) A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts, Anal. Biochem. 154, 536–541.

Pardini R.S. (1995) Toxicity of oxygen from naturally occurring redox-active pro-oxidants, Arch. Insect Biochem. Physiol. 29, 101–118.

Poole H.K. (1970) The wall structure of the honey bee spermatheca with comments about its function, Ann. Entomol. Soc. Am. 63, 1625–1628.

Ruttner F., Enburg H., Kriesten K. (1971) Die Feinstruktur der Spermatheka der Bienenkönigin, Apidologie 2, 67–97.

SAS Institute (1997) SAS/STAT® Software: Changes and enhancements through release 6.12, Statistical Analysis Institute Inc., Cary.

Tolbert N.E. (1978) Peroxisomal redox enzymes, Methods Enzymol. 52, 493–505.

Warholm M., Guthenberg C., von Bahr C., Mannervik B. (1985) Glutathione transferases from human liver, Methods Enzymol. 113, 499–504.

Weirich G.F., Adams J.R. (1984) Microsomal marker enzymes of *Manduca sexta* (L.) midgut, Arch. Insect Biochem. Physiol. 1, 311–321.

White J.W. Jr., Subers M.H., Schepartz A.I. (1963) The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system, Biochim. Biophys. Acta 73, 57–70.

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