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ORIGINAL PAPER

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Electron Paramagnetic Resonance Imaging of Melanin in Honey Bee

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Abstract

Honey bees play a crucial role in the nature by pollinating wild flowers. Over the past years, there has been an increasing concern regarding the honey bee colony decline. Pesticides or environmental effects targeting the biochemistry of insect chitin and cuticle coating may be in part responsible for honey bee pathologies. We here propose the use of electron paramagnetic resonance imaging (EPRI) as a tool to image the melanin–chitin complexes as part of the exoskeleton of the honey bee. EPRI at 9.65 GHz was applied on intact freeze-dried bees. The imaging data were collected on the melanin peak. High-resolution images revealed that this compound is extensively distributed in the periphery of the animal, data consistent with the localization in the cuticle of the bee. While EPR of melanin has been so far explored in the context of melanoma characterization, it may offer new opportunities in research on honey bees and other insects.

Keywords EPR · EPRI · Melanin · Bee · Chitin

Introduction

Honey bees play a crucial role in the nature by pollinating wild flowers. Over the last years, there has been an increasing concern regarding the decline in pollinators, including honey bees. The reason for this decline seems multifactorial as honey bees suffer from the exposure to a variety of stress factors, including the decrease in abundance and diversity of flowers, a decline in suitable habitats, as well as a long-term exposure to pesticides and parasites [1, 2]. Chitin and the cuticle coating are barriers protecting the insect pollinators from the environment, and this protection may be targeted by pesticides or parasites [3–5]. The assessment of damages made to the external envelope of honey bees may benefit from the use of advanced imaging modalities (such as micro-computed tomography) [3]. In this context, we propose here the use of electron paramagnetic resonance imaging (EPRI) to image the melanin–chitin complexes that are present in the exoskeleton of the honey bees. The rationale relies on the presence of an EPR signal from melanin in insect cuticles [6, 7]. Melanin is a mixture of polymers containing indoles and other intermediate products derived from the oxidation of tyrosine. Melanins can be classified into two groups: brown to black pigments termed eumelanin and alkali-soluble yellow to reddish-brown pigments termed pheomelanin [8]. These pigments contain very stable organic semi-quinone free radicals that can be studied by EPR spectroscopy [9, 10]. The EPR spectra of eumelanins are single lines, whereas pheomelanins display a hyperfine splitting due to nitrogen [10]. Previous studies reported the presence of EPR spectra from melanin–chitin complexes in the cuticle of a large variety of insects [6], including in bee corpses [7]. Interestingly, slight changes in EPR spectra were also reported owing to insecticide treatment using cyromazine [6]. The purpose of the present study was to assess the feasibility of applying X-band EPRI on freeze-dried honey bees to image the melanin–chitin complexes from their cuticles.

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Materials and Methods

Honey Bees

Apis mellifera L. honeybees were provided by a local beekeeper at Mons, Belgium. Honey bees were killed by freezing.

Fig. 1 Lyophilized honey bee in a quartz tube for EPR imaging analysis

The bees were extensively rinsed with distilled water. A few bees were dissected with nonmagnetic tools to obtain heads, thoraxes, and abdomens. Of note, we have not observed any radical induced by the crushing. It should be noticed that the mechanic stress is light as the freeze-dried bees are extremely fragile. Other intact specimens were preserved. Bee segments and intact bees were lyophilized. Bee segments were crushed before the analysis and put in a quartz tube of 5 mm diameter. Intact bees were put carefully in a quartz tube of 9 mm diameter (Fig. 1).

Electron Paramagnetic Resonance

EPR measurements were carried out at room temperature using a Bruker Elexsys E540 EPR spectrometer (Rheinstetten,

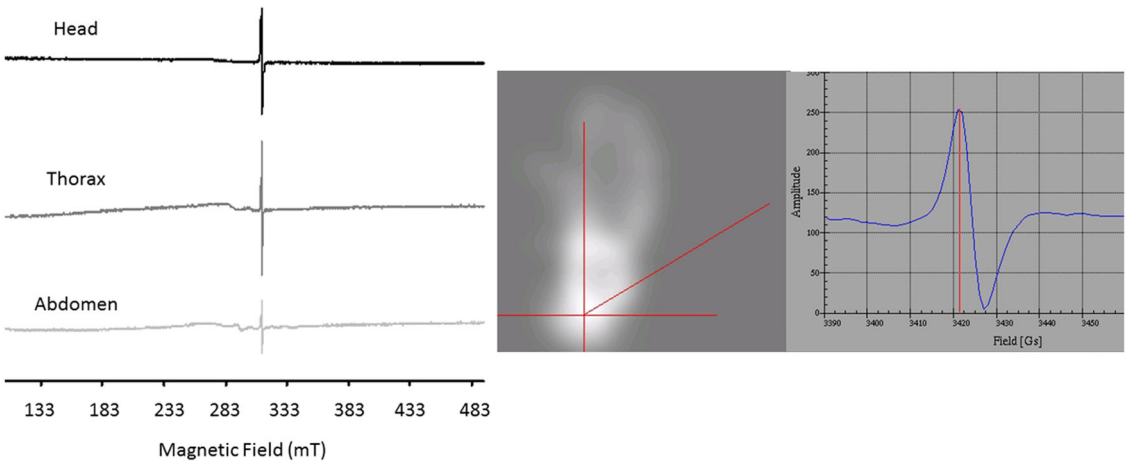

Fig. 2 Left: EPR spectra recorded at room temperature on different segments of the honey bee. Right: EPR spectrum with a narrower sweep recorded on the head of the bee

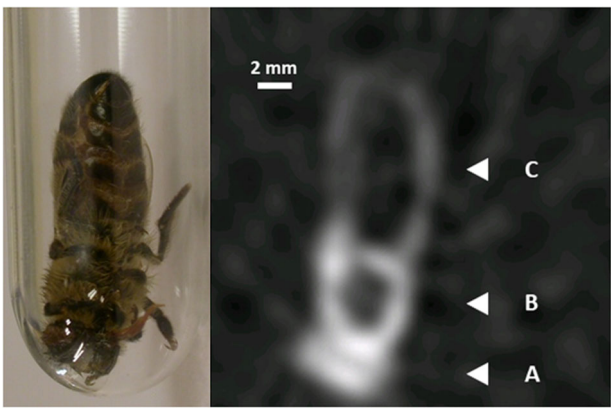

Fig. 3 2D EPR image of the honey bee. The data acquisitions are from the EPR peak recorded at room temperature at $g = 2.004–2.005$. Note that the signal is almost exclusively contained in the periphery of the insect. A: Head. B: Thorax. C: Abdomen

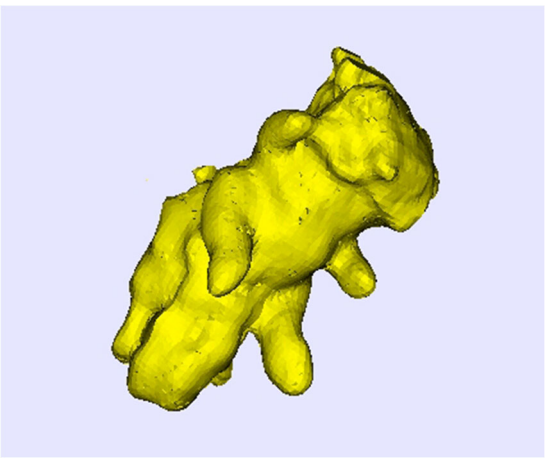

Fig. 4 Volume-rendered image of the presence of free radicals (3D image). The data acquisitions are from the EPR peak recorded at room temperature at $g = 2.004–2.005$. Note the clear delineation of the abdomen, the thorax, the legs, the head, and the proboscis

Germany) equipped with a Super High Sensitivity Probe in combination with a super X bridge operating at 9.65 GHz. The parameters for imaging were: microwave power: 1.046 mW; modulation amplitude: 0.2 mT; modulation frequency: 100 kHz; conversion time: 10.24 ms; time constant: 40.96 ms; sweep width: 33 mT; number of scans: 10; gradient field: 0.49 T/m; field of view = 20 mm; number of projections: 29; number of points: 512; number of scans: 10; pixel size: 0.54 mm; acquisition time: 106 min. The imaging data were collected on the most intense EPR peak located at $g =$ 2.004–2.005. The calculated image resolution was 1 mm. Two- and three-dimensional (2D and 3D, respectively) EPR images were reconstructed using the back-projection algorithm of the Xepr software from Bruker.

Results and Discussion

EPR spectra recorded at room temperature were rather similar from different segments of the bees (Fig. 2). Notably, all spectra displayed an intense simple line at $g =$ 2.004–2.005 with a linewidth of about 0.5 mT. Images were collected on this intense line. The 2D image (presented in Fig. 3) shows that these free radicals are almost exclusively contained in the periphery of the insect. Volume-rendered image of the presence of free radicals (3D image) is presented in Fig. 4. The 3D-EPR image of the external envelope of the honey bee is very well resolved allowing the clear delineation of the abdomen, the thorax, the legs, the head, and the proboscis. In Fig. 5 left, a sagittal slice through the 3D image is presented together with two transversal slices through the abdomen (Fig. 5 center) and through the head of the bee (Fig. 5 right). At the level of the abdomen, free radicals are only visible in the periphery and in the legs. At the level of the head, free radicals are present not only in the periphery but also in the center of the head.

It is interesting to note that other free radicals with g value around 2.002–2.005 can be observed in biological tissues [9, 11], and methods have been developed to confirm the identification of melanin [12, 13]. It is a limitation of our study that such treatments have not been applied, but it will be challenging to keep the insects intact during the identification procedure. When present, the signal coming from free radicals different from melanin is very small in biological samples. Here the characteristics of the EPR signal were consistent with a eumelanin signal, namely, the typical linewidth of 0.5 mT and the localization of the signal in the outer layer of the bee. Moreover, our data are consistent with the spectroscopic data published by others on melanin–chitin complexes present in the cuticle of insects [6, 7]. While this is true for the abdominal part of the honey bee, we cannot exclude the presence of other free radicals in the head of the insects as an EPR signal was also found in the center of the head. At this point, we cannot assess the nature of this radical present in the head. Further studies will be needed to identify these free radicals. In this context, it could be interesting to seek for a possible presence of neuromelanin, as it was recently reported that insects possess the enzymatic machinery to produce neuromelanin in the brain [14].

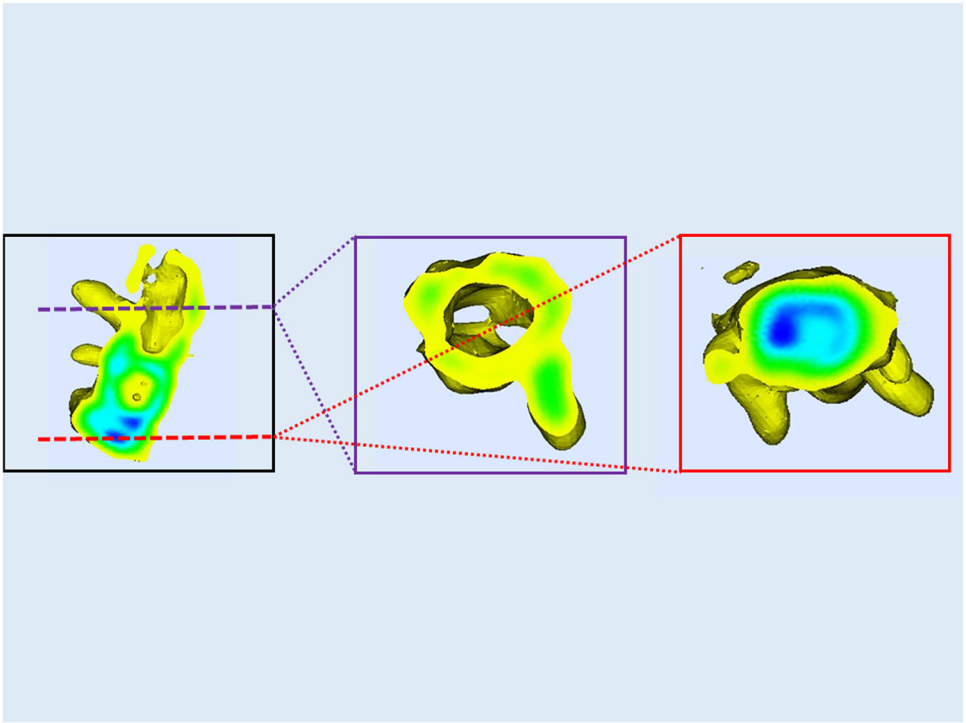

Fig. 5 Volume-rendered 3D images from honey bees. Left: sagittal slice. Middle: transverse slice through the abdomen. Right: transverse slice through the head of the bee

So far, EPRI of melanin has focused on the detection and characterization of melanomas [15–23]. Here we have reported a first example of application of EPRI in insects, namely, honey bee with a focus on the melanin–chitin complexes present in the cuticle. We expect that this report will inspire future research by EPR scientists and entomologists with potential applications to study the effect of environmental factors that may contribute to the degradation of melanin–chitin complexes and may lead to damages in the cuticle of insects. We hope it could especially incite to include honey bees into the species harvesting solar energy in their epicuticle [24], i.e., oriental hornet, and wasps in general. Honey bees exhibit indeed the typical stripped coloration of their abdomen, allowing for a modeling of their surface structures as diffraction gratings. That property is part of the explanation of the tiny radiative quantum yield of eumelanin, which dissipates non-radiatively 99% of the absorbed photon energy. Such conjectures take place in the long quest to understanding the intriguing properties of eumelanin, known to behave as amorphous semiconductors [25].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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