Electron paramagnetic resonance study of honeybee
Apis mellifera abdomens

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Abstract Although ferromagnetic material has been detected in Apis mellifera abdomens and identified as suitable for magnetic reception, physical and magnetic properties of these particles are still lacking. Electron paramagnetic resonance is used to study different magnetic materials in these abdomens. At least four iron structures are identified: isolated Fe$^{3+}$ ions, amorphous FeOOH, isolated magnetite nanoparticles of about $3 \times 10^2$ nm$^3$ and $10^3$ nm$^3$ volumes, depending on the hydration degree of the sample, and aggregates of these particles. A low-temperature transition (52–91 K) was observed and the temperature dependence of the magnetic anisotropy constant of those particles was determined. These results imply that biomineralized magnetites are distinct from inorganic particles and the parameters presented are relevant for the refinement of magnetoreception models in honeybees.

Key words Honeybee · Magnetoreception · Electron paramagnetic resonance · Magnetite

Introduction

Living beings are sensitive to environmental signals. Animals orient themselves during migration or homing using sunlight, skylight polarization, stars, winds, geomagnetic field, etc. (Schmidt-Koenig and Keeton 1978; Kirschvink et al. 1985). Animal species differ one from the other in their use of these environmental signals. Magnetoreception, the animal mechanism to detect the geomagnetic field, is still unknown and its origin remains a complex matter (Wiltshire and Wiltshire 1995). Several hypotheses and models have been postulated to explain different mechanisms for different species (Leask 1977; Gould 1985; Kirschvink and Walker 1985; Presti 1985; Rosenblum et al. 1985; Yorke 1985; Sakaki and Motomiya 1990; Edmons 1992; Grissom 1995; Deutschlander et al. 1999; Shechterakov and Winklhofer 1999). Some of them discuss the nature of the magnetic sensory receptors, suggesting biomineralized magnetic nanoparticles as transducers for the magnetic field information. Lohmann and Johnsen (2000) emphasized that magnetoreceptors have not yet been identified unambiguously in any animal, but it is interesting to point out that, more recently, magnetite has been defined as the magnetoreceptor in rainbow trout (Dielbl et al. 2000). Also recently, Lohmann and Johnsen (2000) emphasized that magnetoreceptors have not yet been identified unequivocally in any animal. As the iron oxide magnetite is a common biomineral (Lowenstam 1981), being widely produced in nature from bacteria (Blakemore 1975) to human beings (Kirschvink et al. 1992; Schultheiss-Grassi et al. 1999), it is a good candidate to be the magnetoreceptor.

Insects are certainly the most diversified and populous group of animals. Wiltshire and Wiltshire (1995) and Vácha (1997) have reviewed the influence of the geomagnetic field on the behaviour of a wide variety of insects. Until now, the largest number of behavioural studies related to the geomagnetic field sensitivity were carried out for the honeybee Apis mellifera (Wiltshire and Wiltshire 1995). Studies on orientation and navigation tasks of these honeybees reveal the use of the sun compass, the skylight polarization, and the geomagnetic field (Gould 1982). Based on the ferromagnetic hypothesis for magnetoreception (Kirschvink 1989), which...
assumes that magnetic particles are involved in magnetoreception, studies of the magnetic properties of the whole honeybee body have shown the presence of magnetite nanoparticles, characterized by its Curie temperature (Gould et al. 1978). Supposing a 100 nm nanoparticle diameters, the magnetization measured is in good agreement with \(15 \times 10^5\) single domains of magnetite nanoparticles found in the front third of the abdomen and aligned transversely to the body axis on their horizontal plane. Later studies on demagnetized bees estimated \(2 \times 10^4\) superparamagnetic particles of magnetite within the 30–35 nm size range (Gould et al. 1980). Different experiments aimed at the localization of sensory magnetic particles in honeybees have been performed and are reviewed in Vácha (1997). Electron microscopy results from magnetic extracts of worker honeybee abdomens (Kirschvink et al. 1993) evidenced two fractions of magnetite nanoparticles: one 15–30 nm in diameter and another with 3–5 nm diameter. These magnetite nanoparticles were only analysed by magnetization measurements.

Electron paramagnetic resonance (EPR) has proved to be a useful technique to identify different structures present in biomineralized magnetic systems. This technique encompasses enough sensitivity to probe inorganic precursors (Berger et al. 1998), as well as biomineralized magnetic material; in addition, the EPR spectra of nonmagnetic structures depend on their size and shape. EPR was used to show the presence of magnetic material in fire ants of Solenopsis spp., possibly related to magnetite (Esquivel et al. 1999), and to analyse magnetic iron oxides in the abdomens of Pachycondyla marginata ants (Wajnberg et al. 2000) and in the abdomens of a honeybee (Takagi 1995). This report is focused on the structural and magnetic parameters of the nanoparticles in the abdomens of the honeybee Apis mellifera obtained from the temperature dependence of the EPR spectra.

The present results contribute to the elaboration and refinement of models on the transduction of the magnetic information in Apis mellifera, since biogenic magnetite is very specific for each species.

**Results and discussion**

Figures 1 and 2 show EPR spectra as a function of temperature for samples A and C, respectively. The spectra obtained are a complex superposition of lines whose composition can vary from one experiment to another. This can be due to the variation of the individual iron contents and/or to sample manipulation and/or to the previous sample histories, as shown by the difference between the spectra of sample A at 280 K before and after cooling (Fig. 1a).

In the low-field region a signal at \(g = 4.3\), assigned to the presence of the Fe\(^{3+}\) ion, can be observed in all spectra. The intensity of this signal strongly decreases with increasing temperatures and it is easily identified in Figs. 1a and 2a.

A six-line structure is noticed in all spectra superimposed on a line at \(g = 2.01\). Arrows (Mn) in Figs. 1a and 2a indicate the peak positions of this component. This signal can be identified with that obtained for the second segment of the abdomen (experimental spectrum in Fig. 1b). The envelope in \(g = 2.01\) is from here on called the background line (envelope spectrum in Fig. 1b). The resonant field and linewidth of this line are temperature independent. A similar EPR signal was observed for marine particles in surface seawater from the English Channel. X-ray diffraction, EPR, Mössbauer spectroscopy, and mass susceptibility measurements of heat-treated particles support the assignment of this EPR signal to the amorphous hydroxide FeOOH (Boughriet et al. 1995). After thermal treatment of the second segments for 20 min at 350 °C followed by 20 min at 450 °C, they were examined (heated spectrum in Fig. 1b). The difference between the spectra of heated and non-heated samples (difference spectrum in Fig. 1b) reveals a relative decrease of the background line and the appearance of a broad line, indicating the conversion of FeOOH to magnetite, maghemite, or hematite. A sharp increase of the narrow \(g = 2\) line is also observed. This line was truncated to have a better resolution of the other components. Furthermore, Takagi (1995) has observed one broad EPR line in only one abdomen of an