High Resolution 2D and 3D EPR Imaging of Melanin in Biological Samples

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Purpose

Electron paramagnetic resonance (EPR) imaging can be described as the spatial and spectral distribution of EPR signals arising from paramagnetic species, in one to three spatial dimensions, using appropriate magnetic field gradients. Melanins are polymeric pigments that contain several free radical centres. At room temperature, the organic semiquinone-type free radical presents a single line (g value of 2.003 to 2.005), with a line width of 5 to 10 Gauss, depending on the melanin type or environment. These features made these free radicals potential candidates for detection and mapping by EPR. To illustrate this approach, we conduct EPR experiments on two types of samples. First, we looked to the possibility to obtain images from B16 melanoma from mice. Second, we looked to the distribution of chitin-melanin and melano-protein complexes in different kinds of insects (bees, ladybirds, woodlouses, scarabs).

Methods and Materials

Melanoma (5 to 8 mm diameter) and insects were freeze-dried. A variety of spatial and spectral-spatial EPR imaging were carried out at room temperature using instrumentation consisting of Bruker ELEXSYS equipped with a Super High Sensitivity Probe in combination with a super X bridge (9.85 GHz).

Results

Illustrative images of the distribution of melanin radicals are presented below (Fig 1, Fig 2, Fig 3, Fig 4). The tumor mass of the melanoma can be well defined by EPR imaging. In insects, 3D EPR imaging revealed that the melanin was present in the cuticle of the insects (chitin-melanin complexes). Besides these free radicals contained in chitin, areas rich in melanin were observed in other locations (such as optical lobes in the brain of bees).

Conclusions

EPR imaging can provide unique information concerning the spatial distribution of the melanin free radicals in biological samples. This technique could be very valuable in oncology to characterize the extent of melanoma in the skin and could potentially be useful, if applied at lower frequencies, to detect melanoma metastases. In natural sciences, this technique could provide detailed information on the areas that are rich in melanin in insects, and may provide a way to characterize the influence of environmental factors (such as insecticides) on the carapace of insects.

Fig 1. 3D EPRI of melanoma

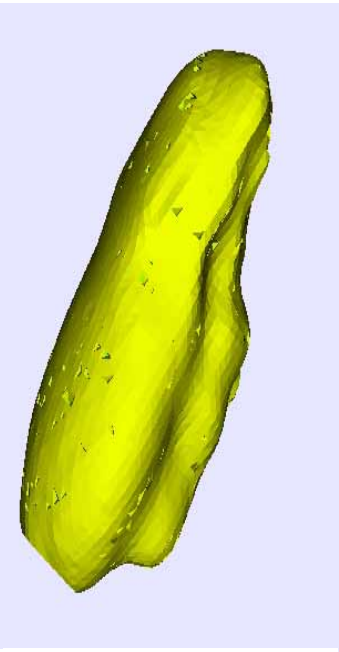

Fig 2. 3D EPRI of scarab's body

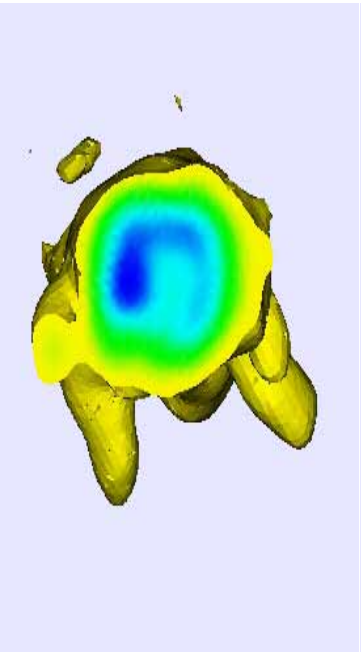

Fig 3. 3D EPRI, section of bee's head

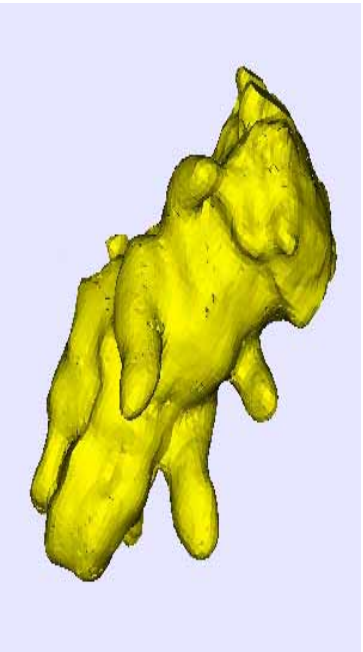

Fig 4. 3D EPRI of bee's body