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研究課題名（和文）磁気感受性を担う細胞内タンパクの分光学的同定と空間特性

研究課題名（英文）Spatially resolved spectroscopic studies of biological magnetosensitivity

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研究成果の概要（和文）：本研究では、光化学反応が磁場から受ける影響をサブミクロンスケールで観測できる新規イメージング装置を開発した。開発した装置を用いて、１ リットルの１０億分の１のさらに１００万分の４という極微小体積中で、フラビンのラジカル対生成、及び微弱磁場によるその反応性の変化の観察することに成功した。更に、本装置では生体内で磁気感受性化学反応が進行している領域の選択的イメージングも可能であり、現在、本手法の生きた細胞への応用を進めている。

本装置の開発により、生物を含む様々な重要な対象において、光化学反応の磁場効果を研究できるようになった。最終的には、動物の持つ驚異的な磁気感受性の機構解明に繋がると期待される。

研究成果の概要（英文）：This research involved the development of a new instrument for imaging the magnetic sensitivity of photochemical reactions on a submicron scale. Using this instrument, radical pairs formed from flavins (molecules believed to be at the heart of biological magnetic sensitivity) and the influence of very weak magnetic fields on their reactivity, were detected in volumes less than 4 millionths of a billionth of a liter. The microscope also allows images to be recorded that display only the regions of a sample where magnetically sensitive photochemical reactions are occurring. This technique is now being applied directly to living cells.

The new imaging microscope developed in this research will enable the study of the magnetic sensitivity of photochemical reactions in a variety of important biological and other contexts, and will help to unlock the secrets of the amazing ability of animals to sense magnetic fields.

研究分野：スピン化学

キーワード：スピン化学　発光分析　クリプトクロム　ラジカル対　検鏡　生物磁気感受能　顕微鏡

１．研究開始当初の背景

The potential magnetic sensitivity of biological systems has been of great interest throughout hundreds of years of history. In the early 1970s, it was demonstrated that chemical reactions could be influenced by weak magnetic fields through the radical pair mechanism (RPM)[1]. Since then, the magnetic sensitivity of chemical processes has been extensively studied[2-4]. However, observation of the magnetic sensitivity of reactions in biology has remained surprisingly challenging[5], despite the generation of pairs of radicals in many biological processes. However, recently there has been much interest in the ability of animals (particularly migratory birds) to navigate using the Earth's magnetic field[6]. It is now believed that the photochemical reactions taking place inside proteins known as cryptochromes involve radical pairs (RP) and are the most likely candidates for this magnetic sensitivity.

２．研究の目的

The goal of this research project was to bring together work by spin chemists on RP reactions in chemical and biological systems and behavioral studies of the magnetic sensitivity of animals, by observing the influence of weak magnetic fields on chemical processes taking place directly within living cells. No techniques exist to measure the photochemistry of flavin reactions and their sensitivity to magnetic fields in a spatially resolved manner. Therefore the objective of this research was to design an instrument that could perform such measurements.

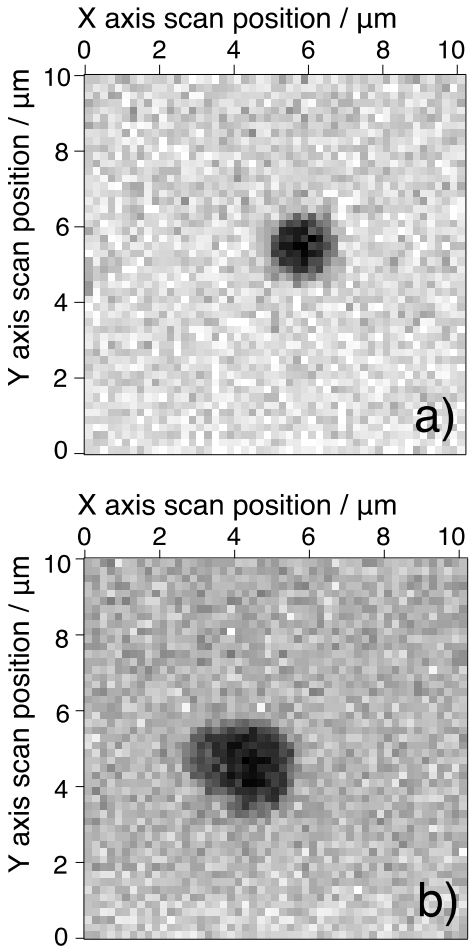
３．研究の方法

This work involved the design, construction, testing, optimization and application of a new kind of microscope, capable of two kinds of measurement: Transient Optical Absorption Detection (TOAD) imaging and Magnetic Intensity Modulation (MIM) imaging. The former technique allows the photochemical reactions taking place in cryptochromes to be monitored inside a very small volume region. This means that the particular structures within a cell (for example the nucleus) can be selectively photoexcited and any RPs produced due to the presence of cryptochrome proteins can be monitored. The effect of a magnetic field on the observed photochemistry in these regions can then be recorded. In the latter technique, the magnetic field sensitivity of the photochemistry is detected directly and so by using this signal to image a sample, only the regions of the sample containing species undergoing magnetically sensitive photochemical processes will appear in the image.

４．研究成果

The microscope was successfully constructed and extensive work was undertaken to fully test and optimize it. The microscope employs a number of techniques to maximize its sensitivity in order to monitor photochemical reactions in real time on very small volumes of sample. To demonstrate the ability of the microscope, Figure 1 shows images obtained from the application of the two new techniques developed during this project: TOAD imaging (Figure 1a) and MIM imaging (Figure 1b), to polymer microbeads of diameter 2.5 – 3 microns in an acidic solution of flavin adenine dinucleotide (FAD), the molecule believed to be responsible for the magnetic sensitivity of cryptochromes. In the TOAD image, the signal used for imaging comes directly from the RP intermediates generated when FAD (concentration 200 μM) is photoexcited with blue light. Thus a greater concentration of RPs gives rise to a brighter pixel. In the MIM image, the signal used for imaging is proportional to the

magnetic sensitivity of the photochemical reaction



taking place. Greater magnetic sensitivity corresponds to a brighter pixel.

Figure 1. a) TOAD and b) MIM images of a 2.5 - 3 µm polymer beads in pH 2.3 buffered FAD solution.

The TOAD imaging method can be applied in two different regimes. The first observes the photochemistry that takes place after a short pulse of blue light is applied. This allows the direct study of microsecond reaction kinetics to be performed. Alternatively, the blue light can be continuously switched on and off at any frequency up to tens of kilohertz. This technique allows cyclic photochemical processes to be studied under continuous illumination, where a photochemical equilibrium is reached – conditions much closer to those observed in nature. In both cases, the instrument proved capable of resolving the effects of very weak magnetic fields (this effect is known as the LFE – low field effect) on the photochemistry of FAD for the first time. Figure 2 shows how the TOAD imaging signal changes with magnetic field at a single pixel positioned away from the microbead in Figure 1. It can be clearly seen that at magnetic fields greater than 1.5 mT, the magnetic field increases the signal, while fields between 0 and 1.5 mT cause a reduction in the size of the signal. This observation is an important one in addressing the potential role of FAD in biological magnetoreception.

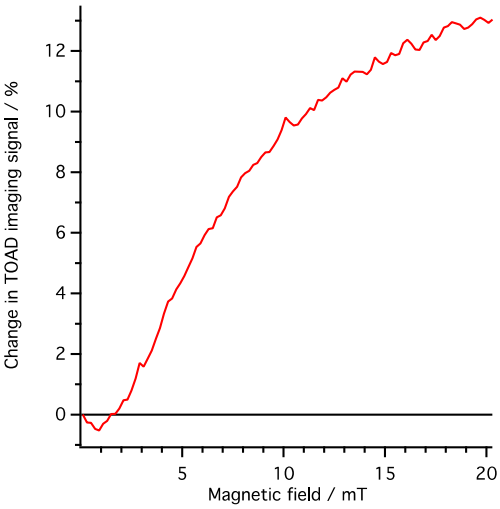


Figure 2. The change in the signal obtained from a single pixel position in Figure 1a with applied magnetic field. The effect of the field is opposite at very weak magnetic fields. This is an example of the low field effect (LFE).

This new instrument and the newly developed techniques of TOAD and MIM imaging are now being applied both to samples of purified cryptochromes protein in solution and of plant and animal cells. These measurements have the potential to help unravel the mystery of biological magnetoreception at the cellular level.

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５．主な発表論文等

〔雑誌論文〕（計　1 件）

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Refereed

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〔その他〕
ホームページ等
http://opes.c.u-tokyo.ac.jp/spinchem/

６．研究組織

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