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研究課題名（和文）Advanced electron spin dynamics measurements in microscopy

研究課題名（英文）Advanced electron spin dynamics measurements in microscopy

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研究成果の概要（和文）：本研究では，ラジカル対(RP)反応での磁場効果の観測に対して新しい微視的アプローチの開発，評価，比較を行った．このようなアプローチは，動物がもつ磁気受容のメカニズムを細胞レベルで解明する上で重要である．そこで，1)観測軸に対して任意方向に磁場を印加し，さらに高速に切替かつ振動する磁場を試料に印加可能にすること，　2)多波長で時間分解光吸収（TROA）信号の同時取得を可能にすることを目標とした．
本研究により，2つの新規顕微鏡が開発された．1つは全反射照明蛍光（TIRF）顕微鏡に基づいており，1)を達成し個々のRPの観測を可能にする．もう１つは，1)，2)を達成するTROAに基づく顕微鏡である．

研究成果の概要（英文）：This project involved developing, evaluating and comparing new microscopic approaches to the observation of magnetic field effects on radical pair (RP) reactions. Such approaches are important in unravelling the mechanism of animal magnetoreception at the cellular level. There were two main goals: 1) to enable the application of rapidly switched and oscillating magnetic fields to the sample along with the ability to apply the magnetic field in any direction relative to the spectroscopic observation axis and 2) to allow the simultaneous acquisition of Time-Resolved Optical Absorption (TROA) signals at multiple wavelengths. The research resulted in the development of two new microscopes. The first is based on Total Internal Reflection Fluorescence (TIRF) microscopy and allows the observation of individual RPs as well as achieving goal 1). The second is a TROA based instrument that achieves goals 1) and 2).

研究分野：　スピン化学

キーワード：　TIRF microscopy　Spin Dynamics　Magnetic Field　Radical Pair　SEMF　RYDMR

１．研究開始当初の背景

Since the development of the Radical Pair Mechanism (RPM) in the 1960s and the observation of Magnetic Field Effects (MFEs) on chemical reactions in the 1970s, electron spin dynamics and their influence on chemical reactivity have been studied extensively using a range of different resonant (e.g. Reaction Yield Detected Magnetic Resonance (RYDMR)[1], Oscillating Magnetic Field Effects (OMFE)[2]) and non-resonant[3] (Magnetic effect on Reaction Yield (MARY), Switched External Magnetic Field (SEMF)) experimental methodologies. Such methods have led to important steps forward in the understanding of a wide range of problems in chemistry, materials science and biology. Many recent studies have focused on the RPM as a potential mechanism to explain animal magnetoreception while others have used magnetic responses in solid state devices to understand and optimize the performance of devices including solar cells and OLEDs. However, until very recently, all experimental approaches have been limited to the study of bulk samples. We have developed a MFEM (Magnetic Field Effect Microscope) for the study of transient radical pair (RP) species based on their electronic absorption spectra (KAKENHI Grant Number 24350002), which for the first time allows the magnetic field sensitivity of biologically relevant RPs to be measured at sub-micron resolution. While powerful, our existing experimental approach allows only for the application of one-dimensional static and audiofrequency oscillating magnetic fields at the sample. In addition, it is limited to making time-resolved optical absorption measurements at a single wavelength.

２．研究の目的

The goal of this research was to investigate a number of experimental approaches that would allow micro spectroscopic studies of RPs to be performed, while providing substantially increased access to the sample, so that more advanced spin manipulation techniques could be performed. These include the application of rapidly switched magnetic fields, oscillating magnetic fields and the ability to align the direction of the magnetic field in any direction relative to the spectroscopic observation axis. The robustness and usefulness of several methodologies were explored and evaluated based on ease of use, sensitivity and reproducibility. In addition, approaches were considered for observing time-resolved optical absorption measurements at multiple wavelengths simultaneously.

３．研究の方法

Our existing micro spectroscope exploits the technique of Time Resolved Optical Absorption (TROA) spectroscopy to detect RPs based on light absorption by one of the pair members. This is a flexible approach which allows observation of a wide range of RPs. Our experimental strategy consisted of two different approaches:

1) TROA based microscopy is the method of choice for the direct observation of RPs. However, it cannot compare in sensitivity to fluorescence based approaches. The technique of objective Total Internal Reflection Fluorescence (TIRF) microscopy also allows free access to one side of the sample as irradiation and fluorescence collection can both be performed by the same objective lens and can be used to observe single molecules. Flavin Adenine Dinucleotide (FAD) is the key molecule involved in the photochemistry of cryptochromes, which are proposed to be the carriers of the animal magnetic compass. In typical flash photolysis experiments, FAD fluorescence is not magnetic field sensitive as

the signal is dominated by the fluorescence of the excited state of the oxidized form of the flavin (which is diamagnetic), which is the state populated under conditions of no irradiation. Continuous irradiation can depopulate the oxidized form and its concentration becomes dependent on the rate of back electron transfer in flavin semiquinone containing RPs, meaning that the fluorescence signal becomes magnetic field sensitive under these conditions. For single FAD molecules, the fluorescence signal under continuous irradiation should also be magnetic field sensitive and should be sensitive to the differing hyperfine states of different molecules. On this basis, we constructed a TIRF based microscope which would allow full access to one side of the sample, enabling the advanced spin manipulation techniques, while also allowing the possibility of observing magnetic field effects (MFEs) on single RPs.

2) Our other primary goal was to be able to perform TROA based microscopy in combination with the advanced spin manipulation techniques. This is more challenging, based on the need for two objective lenses on either side of the sample. Our original approach involved using an objective TIRF-style arrangement and applying the probe beam as an evanescent wave at the sample. The goal was to align high-reflectivity mirrors around the probe beam to allow cavity-enhanced detection of absorption of the probe beam. The pump beam is applied along the center of the objective lens, affording high XY spatial resolution. We tested this approach, but the losses through objective lens were such that the finesse of the cavity was too low to make this approach experimentally useful. We therefore tested an alternative approach by using long working distance objective lenses. This resulted in a small loss of spatial resolution, but allows easy access to both sides of the sample, enabling the advanced spin-manipulation techniques. In additional we developed a technique where the pump and probe beams are supplied to the objective lens off-axis and cross only at the sample region of interest. This approach in principle allows the application of multiple probe beams, enabling simultaneous TROA observation at multiple wavelengths.

４．研究成果

During the project period, different approaches were tested, resulting in the construction of two different microscopes (figure 1). Some example experimental observations from the two instruments are discussed below.

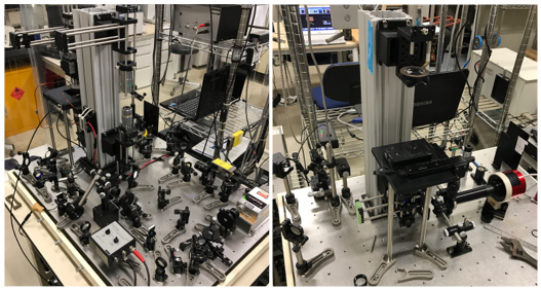

Figure 1. Images of the TROA (left) and TIRF (right) based microscopes developed in this project.

1) Objective TIRF based observation of single FAD molecules.

Our instrument proved capable of observing individual FAD molecules present within a thin layer of solution at the surface of a cover slip (typical thickness is around 100nm). This is an important step in our research and experiments to observe MFEs on these signals are currently underway. As the top surface of the sample is completely open, different magnetic field instrumentation can be mounted to the top side. We

have developed circuitry that can allow a magnetic field of up to around 40 mT to be switched on very rapidly (in less than 10 ns) and held constant for a precisely defined period of time. Alternatively, by mounting both a small rare earth permanent magnet along with a radiofrequency coil, reaction yield detected magnetic resonance (RYDMR) measurements can be performed. We have recently begun work on a new project enabled by this work (KAKENHI Grant Number 17H03005) that will allow a magnetic field to be applied at any three-dimensional angle relative to the optical axis of the microscope. This will allow not only magnetic field effects to be observed but will allow the molecule response to the direction of the applied field to be observed directly.

2) Simultaneous multiple wavelength Transient Optical Absorption Detection (TOAD) microscopy of FAD.

Performing TOAD microscopy at multiple wavelengths can provide important additional information about the kinetics and magnetic field sensitivity of different species involved in photochemical RP reactions. Figure 2a illustrates the approach taken to allow two probe wavelengths (532 nm and 638 nm) to be recorded simultaneously, using identical balanced photodetectors. Figure 2b shows kinetic curves obtained at the two different wavelengths, highlighting the existence of a long-lived signal only at 532nm (which we observed to be highly pH sensitive).

This technique can be expanded to include additional probe wavelengths, although an additional detector and optics are required for each wavelength added.

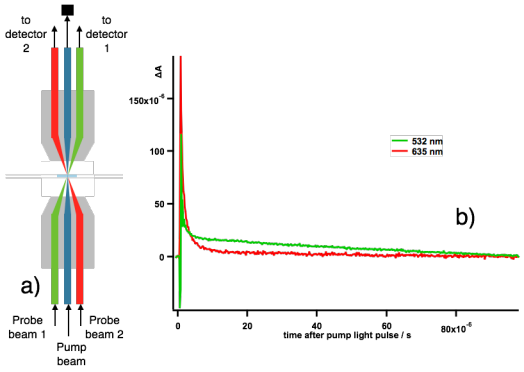

Figure 2. a) Optical configuration for simultaneous multi-wavelength TOAD imaging. b) Transient decays recorded simultaneously at two wavelengths for 200 µM FAD at pH2.3

In summary, two new microscopes were developed and are now being employed for measurements involving advanced spin-manipulation methods. None of the experimental findings have yet been published, but manuscripts are currently being prepared.

1) N. I. Avdievich, E. G. Bagryanskaya, Yu. A. Grishin and R. Z. Dagdeev, *Chemical Physics Lett*ers, 1989, **155**, 141.

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５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔学会発表〕（計　5 件）

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〔その他〕
ホームページ等

http://opes.c.u-tokyo.ac.jp/spinchem/

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