การสร้างภาพของอนุมูลอิสระ ^{14}N ไนตรอกไซด์ ด้วยเทคนิคการสั่นพ้องทางแม่เหล็กของอิเล็กตรอนและโปรตอนแบบลดสนามแม่เหล็กสำหรับอิเล็กตรอน (FC-PEDRI) ที่สนามแม่เหล็กความเข้มต่ำ

Field-Cycled Proton-Electron Double-Resonance Imaging (FC-PEDRI) of a ^{14}N nitroxide free radical at low magnetic field

จิตกร ผลโยญ (C. Polyon)* David J. Lurie (D.J. Lurie)**
วิวัธน์ ยังดี (W. Youngdee)*** ดร.จันทร์เพ็ญ โทมัส (C. Thomas)*** เอียน โทมัส (I. Thomas)***

บทคัดย่อ

การสร้างภาพของอนุมูลอิสระ ^{14}N ไนตรอกไซด์ได้ใช้เทคนิคการสั่นพ้องทางแม่เหล็กของอิเล็กตรอนและโปรตอนแบบลดสนามแม่เหล็กสำหรับอิเล็กตรอน (FC-PEDRI) โดยทำให้เกิดการแทรนซิชันชนิด π แบบอิ่มตัวในปรากฏการณ์การสั่นพ้องทางแม่เหล็กของอิเล็กตรอน (EPR) ได้สร้างขดลวดที่ให้คลื่นวิทยุที่มีการโพลาไรเซชันแบบวงกลมสำหรับ EPR ทำการทดลองเพื่อหาตำแหน่งของการแทรนซิชัน EPR โดยใช้เทคนิคการโพลาไรซ์ทางนิวเคลียร์แบบไดแนมิกแบบลดสนามแม่เหล็กสำหรับอิเล็กตรอน (FC-DNP) กับสารละลายตัวอย่างทดลอง TEMPOL (ระบบ ^{14}N ไนตรอกไซด์) ความเข้มข้น 1 และ 2 mM ที่ความถี่ EPR ในช่วง 45-133 MHz และได้ทำการทดลอง FC-PEDRI ที่สนามแม่เหล็กความเข้มต่ำ (0.3-6.2 mT) สำหรับ EPR โดยรับสัญญาณ NMR ที่สนาม 59 mT สำหรับการทดลองนี้ การแทรนซิชันชนิด π เชิงบวกของ $T_{45\pi}$ มีความสำคัญและมีประโยชน์อย่างมาก เนื่องจากเป็นการแทรนซิชันที่ใช้สนามแม่เหล็กความเข้มต่ำและความถี่ต่ำ ซึ่งมีผลทำให้ลดการดูดกลืนกำลังของ EPR ในตัวอย่างทดลองได้ นอกจากนี้ พบว่าสัดส่วนของสัญญาณต่อสัญญาณรบกวน (SNR) ของภาพผลต่าง (ภาพจากที่มีการกระตุ้น EPR ลบกับภาพจากที่ไม่มีการกระตุ้น EPR) จากการกระตุ้น การแทรนซิชันชนิด π เชิงลบที่ความถี่สูงกว่า (124-133 MHz) จะให้ค่า SNR มากกว่าเมื่อกระตุ้นที่ช่วงความถี่ ที่ต่ำกว่า (45-113 MHz)

* Ph.D. Student, Department of Physics, Faculty of Science, Khon Kaen University.
** Co-Adviser, Bio-Medical Physics, School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK
*** Advisor and Co-Advisers, Department of Physics, Faculty of Science, Khon Kaen University.

ABSTRACT

Field-Cycled Proton-Electron Double-Resonance Imaging (FC-PEDRI) was used to image a ^{14}N nitroxide free radical by saturation of π Electron Paramagnetic Resonance (EPR) transitions. A circularly-polarized EPR resonator was built for use with the FC-PEDRI experiments. To locate EPR π transitions, field-cycled dynamic nuclear polarization (FC- DNP) experiments were performed with 1 and 2 mM TEMPOL solution samples (a ^{14}N nitroxide system) at EPR frequencies in the 45-133 MHz range. FC-PEDRI experiments were performed at low magnetic field (0.3-6.2 mT) for EPR with a proton NMR detection field of 59 mT. The positive π transition of $T_{45\pi}$ is useful in these experiments because the transition is at low magnetic field and low frequency, yielding a lower EPR power deposition in the sample. In addition, the signal-to-noise ratios (SNR) of the difference images (EPR-on minus EPR-off) from the saturation of negative π transitions in the higher frequency (124-133 MHz) were greater than those at lower frequency (45-113 MHz).

คำสำคัญ : FC-PEDRI อนุมูลอิสระ ^{14}N ไนตรอกไซด์ สนามแม่เหล็กความเข้มต่ำ

Key Words : FC-PEDRI, ^{14}N Nitroxide free radical, Low magnetic field

Introduction

Proton-Electron Double-Resonance Imaging (PEDRI) is a powerful technique for imaging free radicals in biological samples or animals. This technique has been developed by irradiation both electron (EPR) and proton (NMR) in a sample. It, also called Dynamic Nuclear Polarization Imaging (DNPI) or Overhauser Imaging (OI), utilizes the Overhauser effect (Overhauser, 1953), involving the observation of the NMR signal of a solvent while irradiating the EPR resonance of a free radical solute: the free radical's unpaired electrons are irradiated by applying irradiation (radiowave or microwave) at the EPR frequency, and the NMR signal is obtained in the usual way by applying pulses of a radiowave at NMR frequency. If there is good magnetic coupling between the unpaired electrons and the water hydrogen nuclei, the EPR irradiation can cause a transfer of polarization from the electrons to the nuclear spins or the solvent nuclei under study, resulting in a large increase in amplitude of observed NMR signal.

Field-Cycled PEDRI or FC-PEDRI has been developed to reduce the problem of high RF power deposition in the sample from the EPR irradiation, without reducing the image signal-to-noise ratio (SNR) (Lurie et al., 1989). In this technique the applied field is switched to a low value for the duration of the EPR irradiation, which is applied at a correspondingly low frequency (typically 50-150 MHz) with correspondingly low absorbed RF power levels. The field is then increased so that the NMR signals can be detected with good SNR.

Field-Cycled Dynamic Nuclear Polarization (FC-DNP) has been also developed to allow the positions and amplitudes of EPR spectral lines to be determined via the Overhauser effect (Lurie et al., 1991).

The aim of this work is to image a ^{14}N nitroxide free radical by saturation of π transitions at low magnetic field.

Materials and Methods

FC-PEDRI experiments were performed with 5.5 ml samples of 1 and 2 mM TEMPOL solutions for the ^{14}N system (T_1 of 938 ms and 669 ms, respectively) at EPR frequencies ranging from 45 MHz to 133 MHz. In the experiments a 5.5 ml $CuSO_4$ solution (which is not a free radical) was used as a reference sample. Moreover, a 38 ml sample of this nitroxide solution was also used to obtain SNR of difference signal images, and enhancement factors.

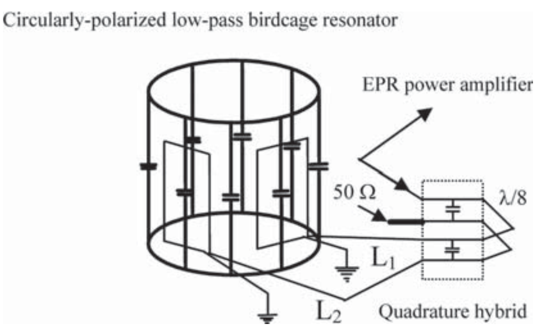

Figure 1 Diagram of a circularly-polarized low-pass birdcage resonator connected with a quadrature hybrid.

In our work, FC-DNP experiments were first performed for obtaining EPR spectra of π transitions, and then FC-PEDRI experiments were continued. All studies were carried out at room temperature using the whole-body sized field-cycling MRI system (Lurie et al., 1998). The eight-leg circularly-polarized low-pass birdcage resonator (Figure 1) was built for use with these experiments. In addition, a solenoid coil tuned to 2.495 MHz was used to detect the NMR signal at the magnetic field of 59 mT.

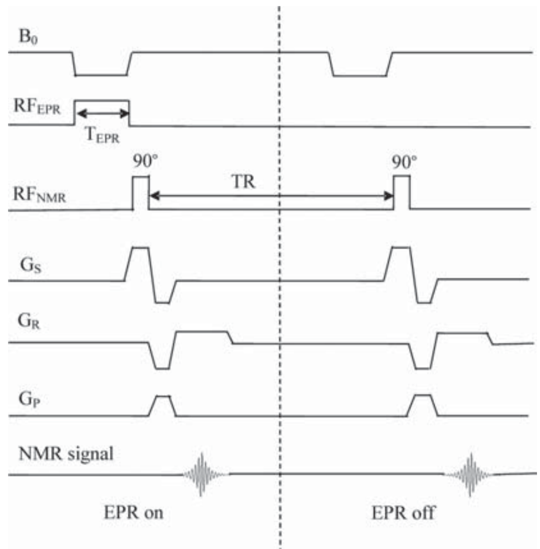

Figure 2 An interleaved FC-PEDRI pulse sequence. A period of EPR irradiation precedes every alternate NMR excitation, allowing 'with EPR' and 'without EPR' images to be collected effectively simultaneously.

FC-PEDRI experiments were performed by using an evolution magnetic field fixed for saturation of a π transition obtained from the previous FC-DNP experiments. An interleaved FC-PEDRI pulse sequence, used with a spin warp for free radical imaging (Figure 2) with EPR irradiation time (T_{EPR}) of 400 ms, detection tme (T_D) of 60 ms, echo time (*TE*) of 26 ms, and repetition time (*TR*) of 1200 ms, was used to allow images with and without EPR irradiation to be collected. Subtraction yielded a difference image which shows non-zero intensity only in regions of the sample containing the free radical of interest.

The images were 64x64 with a field of view (FOV) of 80 mm, a slice thickness of 25 mm, and an EPR power (*P*) of 15 W. By using the same image reconstruction scale factor, the difference signals of each π transition from FC-PEDRI experiments with a 38 ml nitroxide sample (a ^{14}N system) were plotted versus EPR frequency. The Overhauser enhancement factors of these transitions were also plotted and discussed.

Results and Discussion

Figure 3 shows FC-DNP spectra and three 64x64 images (EPR-off, EPR-on and difference) from FC-PEDRI experiments at an EPR frequency of 52.32 MHz with 5.5 ml 3-tube samples of 1 mM and 2 mM TEMPOL (a ^{14}N nitroxide system), and $CuSO_4$ solutions, respectively. Saturation of $T_{45\pi}$ EPR transition was used with T_{EPR} of 400 ms, *TE* of 26 ms, *TR* of 1200 ms, FOV of 80 mm, a slice thickness of 25 mm and an EPR irradiation power of 15 W with the evolution magnetic field of 0.9 mT. The experiments were also performed with other EPR frequencies. The SNRs of the difference images were determined at each EPR frequency. If the FC-DNP spectral peak is deep and narrow, a higher SNR is obtained. The SNR also depends on the concentration of the free radical sample. Thus, SNR of the 2 mM TEMPOL sample was approximately twice as large as that of the 1 mM TEMPOL sample.

To study EPR power deposition in a sample, a bigger sample with 38 ml of 2 mM TEMPOL solution was also used with FC-PEDRI experiments. At an EPR frequency of 45.125 MHz, the measured SNR of difference images from saturation of $T_{45\pi}$ (at an evolution magnetic field of 1.9 mT) and $T_{34\pi}$ (at an evolution field of 2.7 mT) were 2.8 and 6.0, respectively. At an EPR frequency of 91.6 MHz, the SNR of the difference image from saturation of $T_{16\pi}$ at an evolution magnetic field of

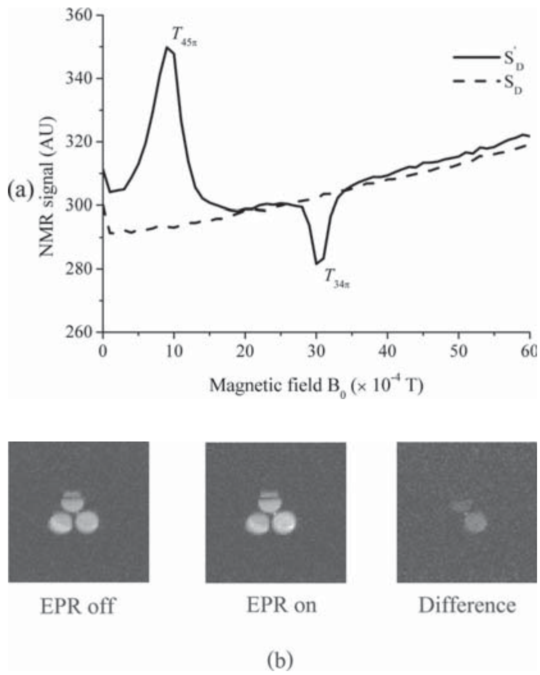

Figure 3 Results of FC-DNP (a) and FC-PEDRI (b) experiments on a sample comprising three 5.5 ml sample tubes containing solutions of 1 mM (upper sample) and 2 mM (lower right) ^{14}N -TEMPOL and $CuSO_4$ (lower left).

FC-DNP spectrum: EPR frequency of 52.32 MHz, T_{EPR} of 400 ms, *TR* of 1200 ms and EPR irradiation power of 15 W

FC-PEDRI images: saturation of $T_{45\pi}$ EPR transition, *TE* of 26 ms, FOV of 80 mm, slice thickness of 25 mm and imaging scale factor of 0.004

The SNR values evaluated from the difference image were 3.1 (1 mM sample) and 6.1 (2 mM sample), respectively.

0.9 mT was 3.8. Similarly, at an EPR frequency of 124 MHz, the SNR of the difference image from saturation of $T_{25\pi}$ at an evolution magnetic field of 3.7 mT was 16.8. For FC-PEDRI experiments with other frequencies of EPR irradiation, the SNR of the difference images are shown in Figure 4.

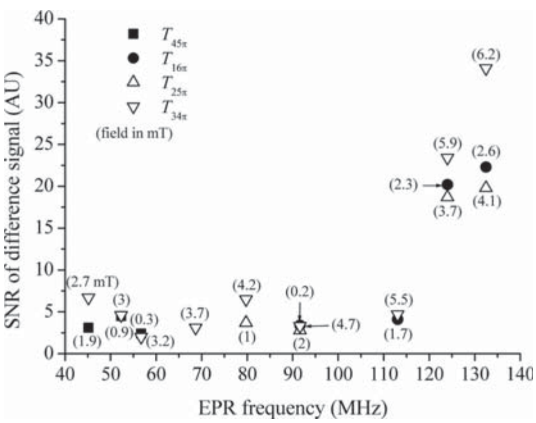

Figure 4 SNR of difference image versus EPR frequency from 64x64 images with the same scale factor of a 38 ml sample of 2 mM TEMPOL solution (a ^{14}N system) by saturation of $T_{34\pi}$ (black-square points), $T_{16\pi}$ (black-cicrcle points), $T_{25\pi}$ (up-triangle points) and $T_{34\pi}$ (down-triangle points) transitions at an EPR frequency 45-133 range with T_{EPR} of 400 ms, *TE* of 26 ms, *TR* of 1200 ms, FOV of 80 mm, slice thickness of 25 mm and EPR irradiation power of 15 W. The numbers in brackets show the magnetic fields used in these FC-PEDRI experiments.

The enhancement factors of the ^{14}N nitroxide free radical at EPR frequency 45-133 MHz range are shown in the Figure 5. Experiments performed at high EPR frequency (124 and 132.445 MHz) show that the SNR values of the difference images were approximately three times as large as the SNR values obtained in experiments with low EPR frequency (45-113 MHz). However, from the figure, enhancement factors of FC-PEDRI images for each π transition were different. The positive enhancement factors, approximately from 1.1 to 1.3, obtained from saturation of the $T_{45\pi}$ transition at EPR frequencies of 45.125, 52.3 and 56.7 MHz are interesting in FC-PEDRI experiments because the sample was imaged at low EPR frequency and low evolution magnetic field (0.3-1.9 mT), yielding lower EPR power deposition in the sample. Furthermore, at high frequency (124 and 132.445 MHz) the sample images were obtained with very low enhancement factors, approximately from -0.2 to -0.1, but nevertheless the high SNR values of the difference images were obtained.

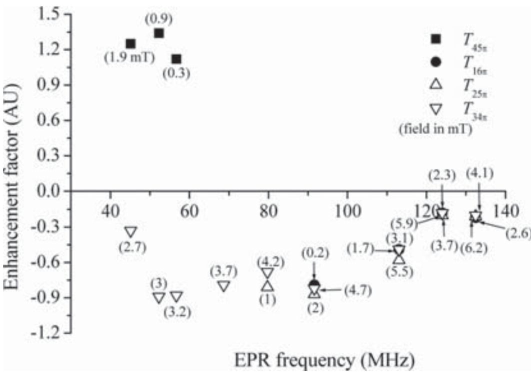

Figure 5 Enhancement factor versus EPR frequency from FC-DNP experiments of a 38 ml sample of 2 mM TEMPOL solution (a ^{14}N system) by saturation of $T_{34\pi}$ (black- square points), $T_{16\pi}$ (black-cicrcle points), $T_{25\pi}$ (up-triangle points) and $T_{34\pi}$ (down-triangle points) transitions at an EPR frequency 45-133 range with T_{EPR} of 400 ms, *TE* of 26 ms, *TR* of 1200 ms, FOV of 80 mm, slice thickness of 25 mm, and EPR power of 15 W. The numbers in brackets show the magnetic fields of these π transitions.

Conclusions

This work focused on experiments with a ^{14}N nitroxide free radical in low magnetic field (0.3-6.2 mT) at low frequency 45-133 MHz range by saturation of π transitions, in order to evaluate the EPR power deposition in the sample. It has been shown that the positive π transition of $T_{45\pi}$ is useful in FC-PEDRI experiments with the ^{14}N nitroxide system, because they can be saturated at low magnetic field and low frequency. With large positive enhancement factors good NMR signals can be obtained. Moreover, the SNR of the difference images from saturation of negative π transitions at higher frequency (124-133 MHz) were greater than those from low frequency (45-80 MHz) for both samples. SNR of a free radical also depends on the free radical concentration. That is, the more its concentration, the more its SNR. To obtain free-radical images, the π transitions of this nitroxide system was saturated by using a circularly polarized EPR resonator with FC-PEDRI experiments. In our work the size of the sample was limited by the small double-resonance RF coil assembly.

It would be interesting to image other nitroxide free radicals such as a ^{14}N nitroxide system, which has a nuclear spin of a nitrogen nucleus equal to 1/2, to see if SNR of its difference images would be improved.

Acknowledgements

This work is supported by the Thai government under a collaboration performed between Khon Kaen University and University of Aberdeen.

References

Overhauser, A.W. 1953. Polarization of nuclei in metals. Phys. Rev. 92: 411-5.

Lurie, D.J., Hutchison, J.M.S., Bell, L.H., Nicholson, I., Bussell, D.M. and Mallard, J.R. 1989. Field-cycled proton-electron double-resonance imaging of free radicals in large aqueous samples. J. Magn. Reson. 84: 431-7.

Lurie, D.J., Mclay, J.S., Nicholson, I. and Mallard, J.R. 1991. Low-field EPR measurements by field-cycled dynamic nuclear polariization. J. Magn. Reson. 95: 405-9.

Lurie, D.J., Foster, M.A., Yeung, D. and Hutchison J.M.S. 1998. Design, construction and use of a large-sample field-cycled - PERDI imager. Phys. Med. Biol. 43: 1877-86.