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Editorial Open Access

Magic Angle Spinning NMR Metabolomics

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Editorial

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive, quantitative, reproducible, untargeted and unbiased method that requires no or minimal sample preparation, and is one of the leading analytical tools for metabolomics research [1-3]. The easy quantification and the no need of prior knowledge about compounds present in a sample associated with NMR are advantageous over other techniques [1,4]. ^{1}H NMR is especially attractive because protons are present in virtually all metabolites and its NMR sensitivity is high, enabling the simultaneous identification and monitoring of a wide range of low molecular weight metabolites.

However, the resolution of the ^{1}H NMR spectra from intact tissues is often poor due to the unwanted line broadenings arising from isotropic magnetic susceptibility variations near boundaries of inter- and intra-cellular structures, residual homo-nuclear proton dipolar coupling and residual chemical shift anisotropy interaction. It is well-known that all of these line broadenings can be eliminated by the technique of Magic Angle Sample Spinning (MAS), where the rotor containing the sample is rotating rapidly about an axis that is inclined at an angle of 54.7356° with respect to the external main magnetic field [5]. The technique, termed as high-resolution MAS (i.e., ^{1}H hr-MAS) [6] using a sample spinning rate of several kHz or more, generates a high resolution ^{1}H NMR metabolite spectrum of intact biological tissue samples with spectral resolution approaching that obtained from standard liquid state NMR on cell and tissue extracts. The unique ability of hr-MAS to study intact tissue samples, i.e., the elimination of extraction process, is a major breakthrough in metabolomics, not only because the extraction takes time and also there is always a concern that some metabolites might be lost during an extraction process. During the last decade, there have been numerous publications in which ^{1}H hr-MAS has been successfully used to study a variety of biochemical processes associated with disease progression and/or the effects of therapies etc. [7].

Typically about 10-60 mg of tissue samples are used for metabolic profiling using the technique of hr-MAS. Recently, a breakthrough has been made in miniaturizing the hr-MAS technique using the concept of Magic Angle Coil Spinning (MACS) [8,9]. MACS uses wireless inductive coupling between the static coil that is used for signal excitation and reception, and a tuned micro-coil that is co-rotating with the sample container. Because the micro-coil is wound directly on capillary sample tube, the sample filling factor is high, resulting in a high sensitivity. MACS is capable of metabolic profiling on tissue sample with mass as small as 0.2 mg. Despite its remarkable success, MACS has the following limitations. (i) A micro-coil (and a fixed capacitor attached to the coil) has to be wound on every sample tube that inevitably increases the cost of the experiment. (ii) MACS favors extremely small sample tube detection by using extremely small diameter of copper wires for winding the micro RF coil due to the factor that the eddy current from a very small rotating micro RF coil is minimized so that high resolution can be obtained. The drawback is that it is not easy to load a tissue sample into a, e.g., 400 μm inner diameter sample tube, thus limiting the throughput of the experiment.

Hr-MAS has now been widely accepted as a powerful NMR metabolic profiling techniques on intact biological tissues samples, and has made indispensable contributions to the success of the rapidly developing field of metabolomics. However, despite its success in metabolomics, hr-MAS is technically challenging due to the use of a sample spinning rate of several kHz or more. These challenges include: (i) Tissue samples contain a significant amount of fluids, mostly H_2O, accounting for more than 90% of the tissue mass. Because quantification is vitally important for NMR metabolomics plus the safety concerns arising from HIV and other potentially infectious disease, a leakage free sealing system at high spinning rate is required for generating quantitative results and for protecting both the experimenters and the expensive MAS probe. Unfortunately, production of a 100% leakage free sample rotor for hr-MAS is challenging due to the large centrifugal force associated with fast sample spinning, especially when the same sample rotor is used repeatedly. (ii) The large centrifugal force associated with fast sample spinning of several kHz or more is destructive to the tissue structure and even some of the cells [10]. As a result, the same sample cannot be used for further analysis by other techniques such as histopathology after hr-MAS. (iii) For large sized biological tissues of ~300 mg or more, it is even more difficult to spin the sample to several kHz. Studying a single organ from a laboratory animal, or larger sample from surgery room can be very important for toxicology assessment and/or disease diagnosis. The application of hr-MAS would be difficult in these cases.

To overcome the technical challenges associated with hr-MAS, my group has recently developed a new approach, called slow-MAS NMR [11-21] that significantly enhances the spectral resolution in excised tissues, organs and live small animals. With one of the methods that we developed based on an existing solid state NMR method originally reported by Antzutkin et al. [22], namely ^{1}H PASS (Phase-Adjusted Spinning Sidebands) [11,12], sample spinning rates as low as 40 Hz can be employed. The method of ^{1}H PASS facilitates non-destructive *ex vivo* studies of excised intact tissues and organs. ^{1}H PASS requires relatively short (from a few minutes to less than an hour) measurement times and offers both high sensitivity and high spectral resolution. Note that a sample spinning rates up to 600 Hz have been reported safe for maintaining the cellular structure of excised prostate tissue [23]. Furthermore, it is easy to keep the fluids inside the tissue without fluid leakage at slow sample spinning rates. Importantly, we have carefully compared the spectral resolution obtained from both the 1H slow-MAS at a spinning rate of 80 Hz and 1H hr-MAS at a spin rate of 2 kHz by using a sample consisting of an intact left lung lobe from a mouse by performing slow-MAS experiment first, followed immediately by hr-MAS on the same sample. We found [20] that the spectral resolution from slow-MAS is significantly higher than that from hr-MAS primarily due to the severe redistribution of the tissue mass at high speed, thus

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the creation of magnetic susceptibility gradient along the magic angle axis that cannot be eliminated by MAS.

We expect that with further development the technology of slow-MAS NMR metabolomics will play a vital role in studying the biochemistry non-destructively in intact biological tissues with variable sample sizes ranging from as small as less than 1 µl to as large as 1 cm^3 or more using a single probe. The small sample volume detection is important for sample limited applications where high spatial sampling for a single type of cells in tissue is required, or where minimally invasive biopsy detection in a laboratory animal or a patient is needed. The large sample volume detection on biological samples with size of 1.0 cm^3 or more is useful for investigating an entire intact organ from a small laboratory animal or tissues from a surgery room in a clinical setting. This will be important for investigating whole organ injury due to a variety kind of insults in laboratory animals, or disease diagnosis in patients.

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