High-resolution Spatially Encoded Intermolecular Double-Quantum Coherence NMR Spectroscopy for Biological Systems

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Target audience

Basic scientists, MRS sequence programmers, researcher scientists who are interested in high-resolution MRS especially in biological tissues.

Purpose

Utilization of the 2D *J*RES technique allows metabolite identifications in disease diagnosis and drug toxicology. However, biological tissues such as muscles, tumors are normally semisolid and, subjected to intrinsic variations in macroscopic magnetic susceptibility. These variations result in field inhomogeneities that broaden lines along the chemical shift dimension in 2D *J*-resolved spectra, concealing information necessary for metabolite identification and obscuring precise *J* coupling measurements. Combined with spatially encoded technique [1], 2D NMR can be applied to monitor biochemical reaction. But the spatially encoded method is intensely affected by the intrinsic inhomogeneity in biological samples. During these years, intermolecular multiple-quantum coherences (iMQCs), originating from distant dipolar interactions among intermolecular spins, have been proven capable for obtaining high-resolution spectra in inhomogeneous fields [2]. Herein, we propose an NMR method called UfiDQC*J*RES combining the advantages of intermolecular double-quantum coherences (iDQCs) and spatially encoded technique to fast record high-resolution 2D *J*-resolved spectra in biological systems.

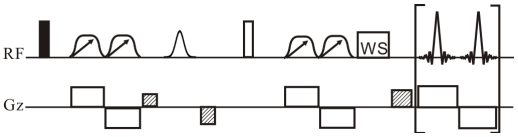

Fig. 1 The UfiDQC*J*RES sequence for acquiring high-resolution 2D *J*-resolved spectra in inhomogeneous fields. "WS" is the water suppression module, the shaped pulses with arrows represent 180˚ adiabatic sweep pulse imparting spatial encoding. Empty (filled) rectangles are 180˚ (90˚) hard pulses. The gradients with slant lines are coherence selection gradients and purge gradient.

Methods

The UfiDQC*J*RES pulse sequence is schematically shown in Fig. 1. A pair of linear coherence selection gradients (CSGs) with an area ratio of 1: –2 is applied. The spatially encoded period and indirect detection period t_1 are divided into two equivalent parts, to form an iDQC evolution period and a *J* coupling evolution period, respectively. A water suppression (WS) module is added prior to acquisition. If the spectrometer reference frequency is set to the resonant frequency of *I* spin (solvent spin), i.e. $\Omega_I = 0$, the resonances will be observed at ($-\pi J_{kl}$, $\Omega_S+\gamma\Delta B+\pi J_{kl}$, $\gamma\Delta B$) and (πJ_{kl}, $\Omega_S+\gamma\Delta B-\pi J_{kl}$, $\gamma\Delta B$), where Ω_S and Ω_I respectively represent chemical shifts of solute and solvent spins, J_{kl} is the *J* coupling constant, γ is the gyromagnetic ratio and ΔB represents field inhomogeneity. Data post-processing of shearing transformation and projection of the 3D spectrum onto the F1-F2 plane can easily move the peaks to sites ($\pm\pi J_{kl}$, Ω_S). Thus, a desired high-resolution 2D *J*-resolved spectrum is achieved.

Results and discussion

Experiments were performed on a Varian NMR System 500 MHz spectrometer. To test the feasibility of UfiDQC*J*RES sequence in biological samples, we peformed an experiment on a sample of *in vitro* brain tissue fitted in a 5-mm NMR tube. Figure 2*a* is the result obtained with 1D water-suppressed proton spectrum without field shimming. The line-width of the water resonance near 4.8 ppm is about 82 Hz, and metabolites in the brain tissues are hardly observable. Figure 2*b* shows the MAS spectrum acquired using a Nano probe. The main metabolites detected in this spectrum can be observed in the UfiDQC*J*RES spectrum (Fig. 2*d*). In the conventional *J*-resolved spectrum in Fig. 2*c*, the invisible chemical shift and *J*-coupling information are retained in UfiDQC*J*RES spectrum, such as lactate (Lac, 1.31 ppm), and alanine (Ala, 1.47 ppm). 27 peaks of 1D MAS spectrum are assigned into 16 metabolites, 22 peaks in the 2D UfiDQC*J*RES spectrum are assigned into 15 metabolites, while, in the conventional 2D *J*-resolved spectrum, it's too obscure for classification. Hence, the influence of field inhomogeneity is eliminated in the 2D UfiDQC*J*RES spectrum. Although the resolution of the peaks in 1D MAS spectrum is better than that in UfiDQC*J*RES spectrum, MAS requires specialized hardware and is not suitable for intact biological tissues and *in vivo* experiment. For example, some vulnerable samples like fish eggs [3] may be damaged due to fast spin, while the UfiDQC*J*RES method provides a complementary way to the MAS technique for some special measurements where samples should be kept intact during the whole detection.

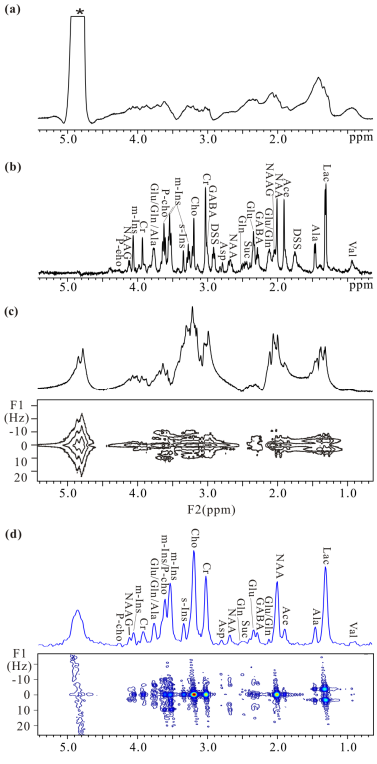

Fig. 2 NMR results of pig brain tissues. (*a*) Conventional 1D spectrum; (*b*) 1D water-presaturated MAS spectrum; (*c*) conventional 2D water-presaturated *J*-resolved spectrum and its 1D *J*-decoupled projection along F2 axis; (*d*) 2D UfiDQC*J*RES spectrum and its 1D *J*-decoupled projection along F2 axis

Conclusion

In summary, the pulse sequence, UfiDQC*J*RES, can acquire high-resolution 2D *J*-resolved spectra from biological systems. This sequence provides an attractive way to characterize biological metabolites in inhomogeneous fields. Spectral resolution enhancement and solvent suppression in this measurement suggest potential applications in *in vivo* studies.

Acknowledgement

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References

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