A high-resolution 2D J-resolved NMR method for intact biological samples

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Target audience

2D *J*-resolved spectroscopy (JRES) is an alternative to 1D NMR for the metabolite analysis due to its ability on separating congested signals along two frequency dimensions.[1] The conventional 2D *J*RES method cannot be directly applied to intact biological samples because of the macroscopic magnetic susceptibility. An effective 2D *J*RES approach, aimed for high-resolution 2D *J*-resolved information on intact biological samples, is presented in this report, and people who perform metabolite analyses in biological systems may be interested.

Purpose

1D ^{1}H NMR spectroscopy is one of the most popular method for metabolomics studies due to rapid spectral acquisition and direct measurements of metabolite concentration.[2] However, two main factors impose limitation on 1D NMR for its applications to intact biological samples. Firstly, the spectral congestion are generally encountered in 1D NMR spectra caused by the limited range of ^{1}H chemical shifts and a large number of peaks from various metabolites. Secondly, 1D NMR study on biological tissues is generally impacted by the field inhomogeneity originated from macroscopic magnetic susceptibility, resulting in spectral line broadening and loss of the desired information for metabolite identification. 2D *J*RES method can provide an effective solution for the spectral congestion in 1D NMR and this method has been applied extensively to metabolite studies in biofluid and tissue extractions.[3] However, the conventional 2D *J*RES method remains unsuitable for the direct measurement on intact biological samples. It is well known that intermolecular multiple-quantum coherences (iMQCs) provide a feasible way to obtain high-resolution spectra in inhomogeneous fields. [4] In this report, an NMR sequence based on intermolecular single-quantum coherences (iSQCs) is introduced to obtain high-resolution 2D *J*-resolved information directly from intact biological samples.

Methods

The iSQC *J*RES sequence for high-resolution 2D *J*-resolved spectrum is shown in Fig. 1. It can be understood intuitively using the raising and lowering operator formalism:

$$I_zS_z \xrightarrow{(\pi/2)_x^I} \frac{1}{4}I^+S_z(t_1) \xrightarrow{(\pi/2)_x} \frac{1}{8}I^+S^+ \xrightarrow{(\pi/2)_x^I} \frac{1}{8}I_zS^+(t_2/2) \xrightarrow{(\pi)_x,\,D_{IS}I_zS_z} \frac{1}{8}S^-(t_2/2+t_3),$$

where *I* spin (corresponding to the solvent) and *S* spins (corresponding to solutes) are spin-1/2 systems and D_{IS} is distant dipolar interactions for iSQCs between solvent and solute spins. And "WS" is the water suppression module for suppressing the strong water signal in biological samples. After the 3D acquisition, the observed signal in a 3D spectrum locates at ($\omega_I+\gamma\triangle B$, $\pm\pi J$, $\omega_{S+}\gamma\triangle B$). A shearing processing on F1 and F3 can be performed to eliminate the field inhomogeneity $\triangle B$ along the F3 dimension, then the location for the observable signal become ($\omega_I+\gamma\triangle B$, $\pm\pi J$, $\omega_S.\omega_I$). When the spectrometer reference frequency is set to the resonant frequency of *I* spin, i.e. $\omega_I = 0$, high-resolution 2D *J*-resolved spectrum can be extracted by projecting the processed 3D data into F2-F3 plane. Although 3D acquisition is needed in the iSQC *J*RES, two indirection dimensions only cover the frequency range of the field inhomogeneity $\triangle B$ and *J* coupling, respectively, thus acquisition efficiency is improved.

A whole zebrafish fitted into a 5-mm NMR tube was used to show the ability of the iSQC JRES method on intact biological samples. The pulse repetition time was 1.5 s, a 4-step phase cycling was applied: the phases for the first RF pulse, the third RF pulse, and the receiver were (*x, -x, x, -x*), (*x, x, -x, -x*), and (*x, -x, -x, x*), respectively. 12 × 25 × 600 points were acquired with spectral widths of 150 Hz × 55 Hz × 5000 Hz (F1 × F2 × F3) in 30 min. For comparisons, a traditional 1D NMR and 2D *J*RES experiment was also performed under the same condition. All experiments were performed at 298 K using a Varian NMR System 500 MHz spectrometer, equipped with a 5 mm indirect detection probe with 3D gradient coils.

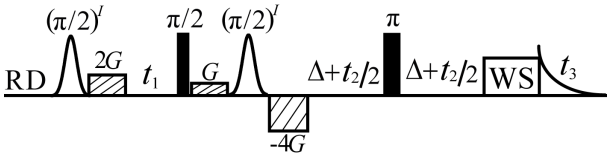

Fig.1 Pulse sequence for high-resolution 2D *J*-resolved spectra directly from intact biological samples.

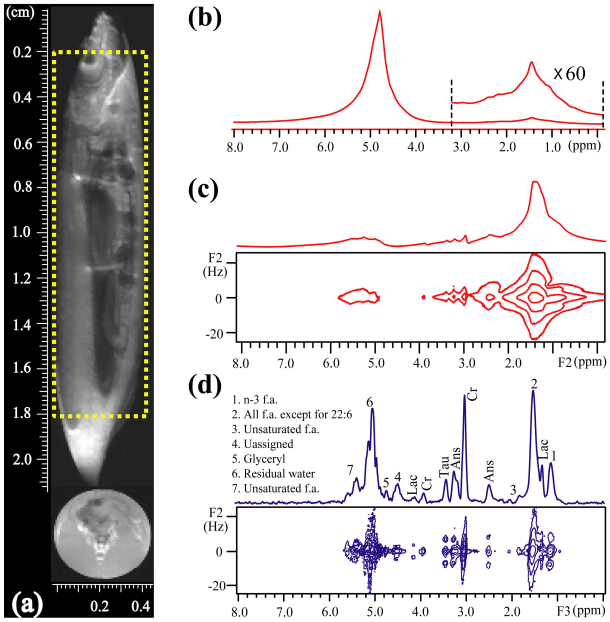

Fig.2 NMR results for a whole zebrafish fitted in a 5-mm NMR tube. (a) Sagittal and axial spin-echo images with the marked detection region. (b) conventional 1D spectrum and the expanded region for metabolites; (c) conventional 2D *J*RES spectrum; (d) 2D iSQC *J*RES spectrum with assigned metabolites.

Results and discussion

The comparison results on a whole zebrafish acquired from the conventional NMR sequences and the iSQC *J*RES sequence are shown in Fig. 2. Figure 2a is the sagittal and axial spin-echo images of the fish. Figure 2b is a conventional 1D NMR spectrum of the intact fish and it shows the field circumstance for this study. For the conventional 2D *J*-resolved spectrum (Fig. 5c), the water signal is suppressed, however, the inhomogeneous line broadening still influences the spectral analysis. It is difficult to assign metabolites correctly and measure *J* coupling constants accurately. The iSQC *J*RES method provides an alternative way for the *J*-resolved detection on intact biological samples. From the resulting 2D iSQC *J*RES spectra of the zebrafish (Fig. 2d), it can be seen that spectral resolution is greatly enhanced and the line-width of the Cr peak at 3.19 ppm is 20 Hz. Compared to the conventional 2D *J*RES spectrum in Fig. 2c, much more signal peaks are observed in the 2D iSQC *J*RES spectrum in Fig. 2d. Five peaks from the fatty acids (f.a.) and four eight peaks from other small metabolites are observed in Fig. 2d, and the related *J* coupling constants are also accessible. It's clear that the iSQC *J*RES method holds the ability to improve spectral quality on 2D *J*-resolved spectra and provides a useful tool for studying metabolites in intact biological samples.

Conclusion

Here, we present a NMR acquisition method for achievement of high-resolution 2D *J*-resolved information directly from intact biological samples. Compared to the conventional *J*RES technique, high-resolution *J*-resolved information can be obtained by using the proposed method, useful for metabolite analyses. This proposed method is merely based on pulse sequence design and can work on standard NMR spectrometers without any hardware requirement, providing a convenient and complementary way for metabolite studies on intact biological samples.

References

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