High-resolution ^{1}H magnetic resonance spectroscopy of whole fish, fish eggs and fish muscles via intermolecular multiple-quantum coherence

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Target audience

Target audiences of the present study are basic scientists and scientists who are interested in NMR study on biological tissues.

Purpose

Fish which is rich in unsaturated fatty acids, protein, calcium, phosphorus, iron and vitamins, etc., is one of the most popular foods. Both lipids and small metabolites are related to the nourishment of fish [1]. Therefore, it would be of great value if the components can be identified and quantified. However, fish tissues are in semisolid phase which may be subject to intrinsic variation in magnetic susceptibility over the sample volume. Their high resolution (HR) NMR spectra could not be obtained by conventional methods. Nowadays, there are normally two methods for HR NMR spectra of fish tissues: liquid NMR of tissue extracts and HR magic angle spinning (HR MAS) NMR of *ex vivo* tissues. Although these two methods are popular, there are some disadvantages. Recently, Hadamard encoding technique [2] was introduced to intermolecular multiple-quantum coherence (iMQC) NMR spectroscopy to obtain 1D HR liquid NMR spectra in inhomogeneous magnetic field [3]. The new method can provide an HR spectrum directly and non-invasively through 1D acquisition within a relatively short acquisition time. In this paper, the feasibility of *in vivo* HR iMQC spectroscopy of this new method was demonstrated through experiments on *ex vivo* fish muscle tissue and fish eggs and *in vivo* whole fish. For comparison, the HR MAS was also applied.

Methods

All iMQC experiments were performed on a 499.74 MHz NMR spectrometer at 298 K using a 5 mm indirect detection probe equipped with 3D gradient coils. The intact salmon muscle, shishamo smelt eggs and Siamese algae eater were stuffed directly into a 5 mm NMR tube without any pretreatment. An 8-order Hadamard matrix for the Hadamard-encoded iMQC pulse sequence [3] was used. The total acquisition time is within 18 min. 1D HR MAS spin-echo experiments were performed on a Bruker AVANCE^{III} 400.19 MHz spectrometer at 298 K equipped with a 4 mm MAS rotor. The sample was spun along the magic-angle (54.7°) direction at a rate of 8 kHz. For the whole Siamese algae eater, it is necessary to dissect it into small fillets before fitting into the MAS rotor.

Results and discussion

The ^{1}H NMR spectra of intact salmon muscle tissue and shishamo smelt eggs are shown in Figures 1 and 2. Because of the intense and broad water signal and inhomogeneous line broadening resulted from magnetic susceptibility gradients among the fish muscle tissue and fish eggs, hardly any spectral information can be obtained (Figures 1A and 2A). Hadamard-encoded iMQC 1D spectra acquired under the same circumstances are shown in Figures 1B and 2B. For comparison, HR MAS spectra are shown in Figure 1C and 2C. It can be found that most of fatty acids as well as small metabolites are observable in the iMQC spectra except glyceryl (4.30 ppm) and unsaturated fatty acids (5.34 ppm) which may be due to the solvent suppression since these two resonances are adjacent to the big water peak. 50 times magnified photos of fish eggs before and after MAS taken by electronic microscope are shown in Figure 2D. As we can see, the eggs which are globular and plump before MAS become shriveled and bleached after MAS. The results of the whole Siamese algae eater was investigated by the Hadamard-encoded method are shown in Figure 3. The anatomic spin-echo images were acquired for location (Figure 3A). Similar to the above two studies, most small metabolites and fatty acids are observable in *in vivo* HR spectrum. However, for the spectrum of the whole Siamese algae eater (Figure 3C), there are other peaks missed, such as fatty acids (2.03 ppm), creatine (3.95 ppm), lactate (4.14 ppm) and histidine (7.13 and 8.12 ppm). This may be due to the more complex circumstance caused by magnetic susceptibility variation between the fish and the air in the interspaces in the NMR tube and the intrinsic magnetic susceptibility variation in the fish itself among muscle tissues, bones, viscera and fish scale etc. And the low concentration of some small metabolites is another possible reason.

Conclusion

The experimental results verify the feasibility of the Hadamard-encoded iMQC technique in detecting structured semisolid-state samples comprised of complex components and even whole organism. Compared to the HR MAS, the resolution and sensitivity of resulting spectra are not as high as MAS. However, the iMQC method is non-invasive. Compared to the conventional iMQC method [4], the introduction of Hadamard encoding technique saves acquisition time.

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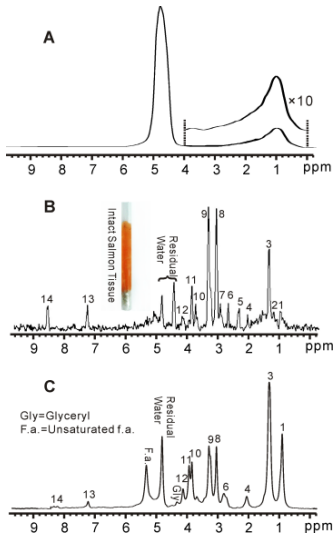

Figure 1. Intact muscle tissue (A) Conventional 1D spectrum. (B) 1D *ex vivo* iMQC spectrum. (C) 1D HR MAS spectrum.

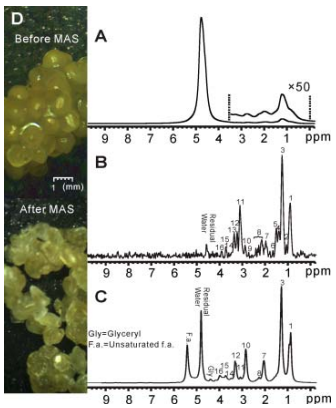

Figure 2. Shishamo smelt eggs. (A) Conventional 1D spectrum. (B) 1D *ex vivo* iMQC spectrum. (C) 1D HR MAS spectrum. (D) 50 times magnified photos of eggs before and after MAS.

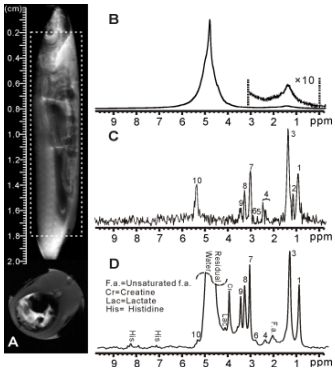

Figure 3. Siamese algae eater. (A) Sagittal and axial spin-echo images. (B) Conventional 1D spectrum. (C) 1D *in vivo* iMQC spectrum. (D) 1D HR MAS spectrum.