Ex vivo quality-related changes in fish muscle and fish eggs during storage by high-resolution ^{1}H magnetic resonance spectroscopy via spatial encoding intermolecular single-quantum coherence

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Target audience

The target audience of present study is basic scientists and scientists who are interested in NMR study on biological tissues and quality control of fish.

Purpose

The high-resolution (HR) NMR spectroscopy with fine NMR spectral information has been a powerful tool for a noninvasive, rapid and accurate composition analysis on solution samples. However, tissues are in semisolid phase, the HR spectra cannot be obtained by conventional NMR spectroscopy. Nowadays, liquid NMR of tissue extracts and HR magic angle spinning (MAS) NMR are widely used for HR NMR spectra of tissues. Although these two methods are popular, there are some disadvantages. For example, complicated pretreatments are necessary for the extraction method, a specialized Nano or solid-state rotor must be equipped for HR MAS NMR and some vulnerable samples may be damaged as a result of fast spin. Intermolecular multiple-quantum coherence (iMQC) is originated from the dipole-dipole interactions, which are effective within a range of 5 ~ 500 μm that is far smaller than a typical sample dimension. Therefore, iMQC has been applied in inhomogeneous biological tissues for HR NMR spectra. However, the HR NMR spectra by iMQC is obtained from a 2D acquisition, dozens of minutes is normally required, which is time-consuming. Recently, a new UF-IDEAL III sequence which is based on intermolecular single-quantum coherence (iSQC) and spatial encoding for ultrafast acquisition (named as UF iSQC) is proposed by our group [1] (Fig. 1). By this method, not only is the HR spectroscopy recovered in inhomogeneous field, but also the acquisition time is greatly reduced. This reminds us to introduce the UF iSQC method into the investigation of quality-related changes of fish tissues. In this work, the metabolites changes of salmon muscle and shishamo smelt eggs during storage are explored.

Methods

All iMQC experiments were performed on a 500 MHz NMR spectrometer at 298 K using a 5 mm indirect detection probe equipped with three dimensional gradient coils using the UF iSQC method (Fig. 1). The intact salmon muscle and shishamo smelt eggs were stuffed in 5 mm NMR tubes without any pretreatment, respectively. The spectra of fish muscle and fish eggs were acquired with the same experimental parameters at two different time-points, 0.5 hour (fresh status) and 7 days (saved in 3 degree refrigerator, decayed status) within 5 minutes.

Results and discussion

The experimental results of the intact fish muscle are shown in Fig. 2. Fig. 2a is the conventional 1D spectrum, obviously, the peaks are overlapped and no useful spectral information can be obtained except the enormous water signal due to the intrinsic variation in magnetic susceptibility over the sample volume. The UF iSQC spectrum shown in Fig.2b was acquired from the same sample as that in Fig. 2a. The spectral resolution is improved dramatically and most of the metabolites peaks appear and become distinguishable. The UF iSQC spectrum of the decayed salmon muscle was also obtained with the same experimental parameters (Fig. 2c). The assignments of the peaks are shown on the left of Fig. 2b [2]. By comparison, it has been found that the relative intensities of the peaks are changed a lot. There into, acetate increases dramatically, and the glutamate/glutamine which were absent in Fig. 2b are shown in Fig. 2c. It has been reported that acetate and glutamate will accumulate during the spoilage of fish [2]. In contrast, histidine is obviously decreased, which is also in accordance with the literature that histidine concentration was observed to decrease, as a consequence of decarboxylation leading to histamine [2]. The experimental results of the *ex vivo* shishamo smelt eggs are shown in Fig. 3. As the same as the results of Fig. 2, the HR spectra of fresh and decayed fish eggs are obtained by UF iSQC (Fig. 3b and 3c), while conventional 1D spectrum is powerless (Fig. 3a). The water signal is effectively suppressed and most of the fatty acids signals as well as the small metabolites are observable. Similarly, acetate is also elevatory. Besides, ancerine increases dramatically and the peak of fatty acids (1.55 ppm) is appeared which may be related to the oxidation of unsaturated fatty acids.

Conclusion

In this work, UF iSQC method was employed to fast obtain HR 1D NMR spectra of intact fish tissues. Furthermore, the quality-related changes during storage were analyzed. The UF iSQC provides a feasible fast screening method for quality control of semisolid food and inspections on biological tissues *in vivo*.

Acknowledgement

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References

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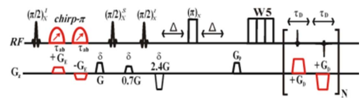

Figure 1. UF-IDEAL III pulse sequence. The semi-ellipses with sloping arrows indicate adiabatic frequency-swept 180 pulses. The gradient GP prior to acquisition is adjustable to set echo peaks in the middle of detection period. The 90 solute-selective pulse is constitutive of a 90 hard pulse and a 90 solvent-selective pulse with opposite phase.

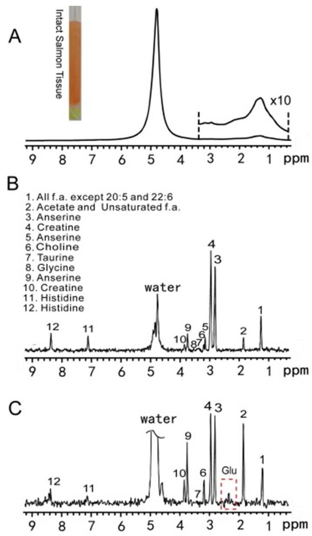

Figure 2. ^{1}H NMR spectra of intact muscle tissue from Atlantic salmon. (A) Conventional 1D spectrum. (B) and (C) 1D *ex vivo* UF-iSQC spectra of fresh and decayed salmon muscle, respectively.

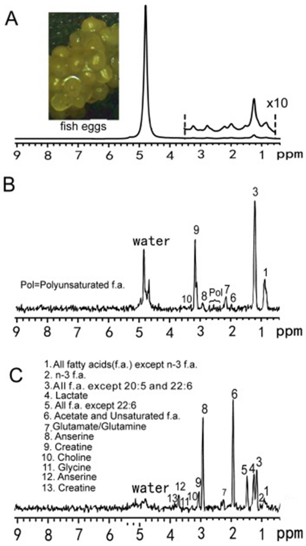

Figure 3. ^{1}H NMR spectra of shishamo smelt eggs. (A) Conventional 1D spectrum. (B) and (C) 1D *ex vivo* UF-iSQC spectra of fresh and decayed shishamo smelt eggs, respectively.