Determination of fatty acid profile of intact fish by intermolecular double-quantum coherence ^{1}H-NMR spectroscopy

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Target audience

The target audience of present study is basic scientists and scientists who are interested in NMR study on fatty acids of biological tissue especially fish.

Purpose

The smoothness of the texture and flavor of salmon muscle are greatly influenced by the fat content, meanwhile, high content of unsaturated fatty acids can reduce arterial diseases and have a positive effect on brain and nervous systems. Consequently, it will be of interest to develop an analytical method that permit a simple, time and resource-saving analysis of lipids in fillet samples and *in vivo*. There are mainly two methods for NMR spectra of fish tissues: extraction NMR and magic angle spinning (MAS). Although these two methods are popular, some limitations exist. For example, chemical extraction is an environment-unfriendly, resource-consuming technique that involves solvents such as methanol and chloroform. Moreover, lipid oxidation may occur during the sample preparation. For MAS, a specialized rotor must be equipped. These drawbacks lead to the development of a method as supplement for extraction NMR and MAS. Intermolecular double-coherence (iDQC) is originated from the dipole–dipole interaction between spins within a specific distance called dipolar correlation distance d_c. The d_c, which is typically 10 μm to 10 mm, is defined as $d_c = \pi/(\gamma * GT)$, where γ is the gyromagnetic ratio and GT is the area of the coherence selection gradient (CSG). Therefore, iDQC signals only from fat molecules can be obtained by exciting the methylene protons and controlling the strength of CSG, if d_c is adjusted to approximate the size of a fat molecule. Meanwhile, signals from metabolites and water which are around fat molecules can also be filtered out theoretically.

Methods

All iDQC experiments were performed on a 500 MHz NMR spectrometer at 298 K using a 5 mm detection probe using the iDQC method, namely IDEAL-II.[1] In order to show the feasibility in *in vivo* studies, a localization module was introduced into IDEAL-II. The intact salmon muscle and zebra fish were stuffed in a 5 mm NMR tube without any pretreatment. All the iDQC spectra are obtained within 10 minutes. To compare and confirm the validity of iDQC experiments, the extraction NMR is also applied.

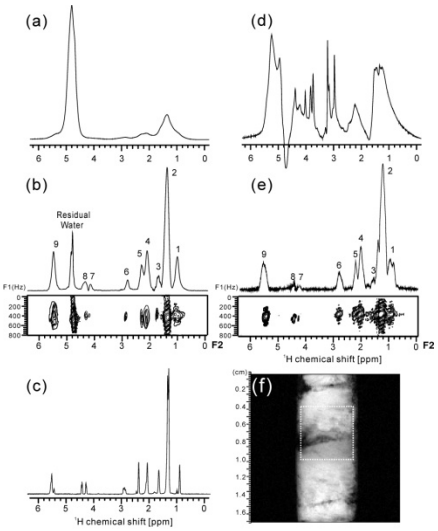

Figure 1. ^{1}H NMR spectra of salmon muscle. (a) Conventional 1D proton spectrum, (b) iDQC spectrum, (c) Conventional 1D proton spectrum of lipophilic extracts, (d) PRESS spectrum with VAPOR module obtained from a voxel of 4×4×4 mm3, (e) Localized iDQC spectrum from the same voxel as that in Fig.1d, (f) Sagittal spin-echo image.

Results and discussion

The experimental results of the intact salmon muscle are shown in Fig. 1. Due to the intrinsic inhomogeneity of salmon muscle, the conventional proton spectrum, as shown in Fig. 1a, cannot resolve well. The unsaturated fatty acids signals are covered by the strong water and methylene signals. By contrast, the accumulated projection 1D spectrum of the 2D iDQC spectrum leads to a dramatic resolution enhancement. The half linewidth of the methylene resonance at 1.3 ppm is reduced from 220 Hz to 82 Hz, and more peaks can be identified and resolved (Fig. 1b). Besides, the water signal is suppressed naturally in the iDQC spectrum without any water suppression module as expected. The conventional 1D proton spectrum of lipophilic extracts of the salmon muscle as shown in Fig. 1c confirms the result of iDQC. Fig. 1f shows the sagittal image of salmon muscle, the localized voxel is denoted by a dashed box. The PRESS spectrum with the variable power and optimized relaxation delays (VAPOR) module is shown in Fig. 1d. Both the low resolution of the spectrum and the overlap with metabolite signals seriously interfere with the identification of fatty acids. In contrast, a high resolution pure fat spectrum is recovered by the localized iDQC method (Fig. 1e). The localized spectra and images from a postmortem study of a whole zebra fish are shown in Fig. 2. As expected, the resolution enhancement spectrum (Fig. 2d) is recovered from the intrinsic magnetic inhomogeneity of the fish (Fig. 2b and 2c) by localized iDQC method.

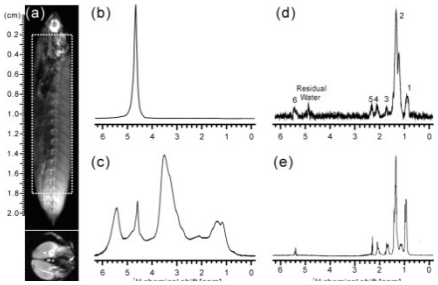

Figure 2. Localized ^{1}H NMR spectra of whole zebra fish. (a) Sagittal and axial spin-echo image, a localized voxel with the size of 4 × 4 × 16 mm3 is denoted by a dashed box; (b) PRESS spectrum obtained from the voxel shown in Fig. 2a, (c) PRESS spectrum with VAPOR module obtained from the same voxel as that in Fig. 2b, (d) Localized iDQC spectrum from the same voxel as that in Fig. 2b, (e) Conventional 1D proton spectrum of lipophilic extracts.

Conclusion

In this work, iDQC method is employed to fast obtain high-resolution NMR pure fatty acids spectra of fishes without pretreatments. This method can not only recover the spectral information of fatty acids concealed by inhomogeneous line broadening, but also filter out signals from small metabolites and water.

Acknowledgement

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References

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