In vivo High-Resolution Magic Angle Spinning Proton MR Spectroscopy of Small Whole-Model Organism C. elegans

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Introduction–Metabolic profiling is an emerging field of postgenomic sciences, which focuses on identifying and quantifying low molecular weight compounds (metabolites) to obtain information at a molecular level and to investigate the response of a living organism to pathophysiological stimuli (1). This can be achieved by simultaneous quantification of metabolites using spectroscopic methods such as nuclear magnetic resonance (NMR) or mass spectrometry (MS) (2,3). Here, we capitalize on recent developments in solid-state NMR that allow the acquisition of highly resolved 1H spectra of metabolites from inhomogeneous materials such as biopsies (4,5). We have recently shown that a whole-organism *in vivo* NMR-based metabolic profiling strategy can be used towards the investigation of the effects of trauma, innate immunity and aging on the small fruit fly *Drosophila melanogaster* (6). In the present study, we apply a strategy using ^{1}H HRMAS NMR spectroscopy of whole-model organisms, in this case the small worm *Caenorhabditis elegans (C. elegans),* which has been used extensively in studies of aberrant metabolism (7). This is the first demonstration of ^{1}H HRMAS NMR in living worms. Results with worms fixed with formaldehyde have been reported previously (8).

Materials and Methods– *C. elegans* preparation: At the fourth larval stage (L4 stage), *C. elegans* worms were washed free of OP50 *E. coli* bacteria from normal growth media (NGM) Agar plates using modified phosphate-buffered saline (M9 buffer). Worms were washed with M9 four times and allowed to settle by gravity at room temperature. Approximately, 16,000 worms (3 dishes) were used to fill a 4mm HRMAS rotor (80 µl) (Fig.1). **NMR:** *C. elegans* were kept at 20ºC while in the spectrometer. The temperature was controlled at 20ºC by a BTO-2000 unit in combination with a MAS pneumatic unit (Bruker). All ^{1}H HR-MAS experiments were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HR-MAS probe (Bruker). The worms were placed into 4mm zirconium oxide (Zirconia, Bruker) rotor with spherical inserts (Fig. 1). 10 µl D2O (deuterium lock reference) containing 10 mM TSP (trimethylsilyl propionic-2,2,3,3-d4 acid, Mw=172, δ=0ppm, external chemical shift reference) was added to the rotor with the sample. To avoid contact between worms and D2O, parafilm was inserted at the bottom of the rotor. To avoid side bands due to imperfect water suppression, the MAS rotation frequency was stabilized at 3.5 ± 0.001 kHz by a MAS speed controller. We have performed one-dimensional experiments by using pulse sequences implemented in the Bruker software: i) a composite pulse sequence (zgcppr) with 1.5 s water presaturation during the relaxation delay, 8 kHz spectral width, 32k data points, 32 scans; and ii) a water-suppressed spin-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence (cpmgpr) with 1.5 s water presaturation during the relaxation delay, 1 ms echo time and 20-100 ms total spin-spin relaxation delay (2nt), 8 kHz spectral width, 32k data points, 64 scans. All free induction decays were multiplied by an exponential function equivalent to a 0.5 Hz line-broadening factor before Fourier transformation for a size of real spectrum of 32k data points.

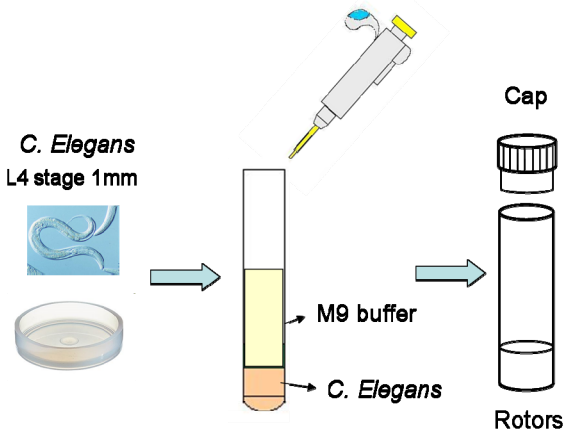

Figure 1. At the 4th larval stage (L4 stage), *C. elegans* worms were washed free of *E. coli* bacteria from Agar plates using phosphate-buffered saline (M9 buffer) four times and allowed to settle by gravity at room temperature. Approximately 16,000 worms were used to fill a 4mm HRMAS rotor.

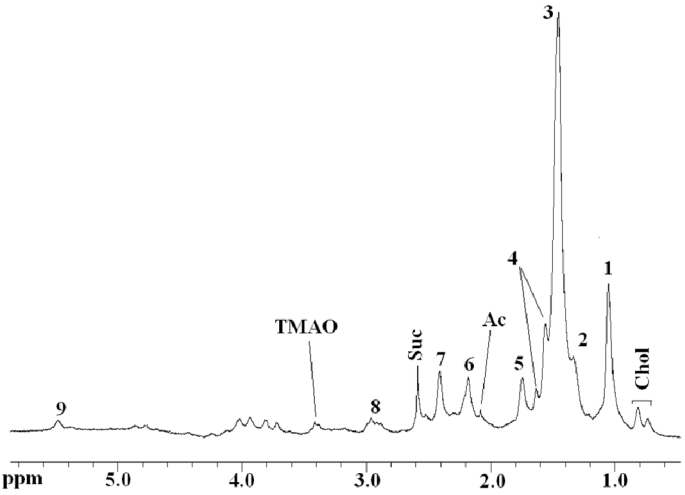

Figure 2. Representative 1D NMR spectrum from living *C. elegans*. Lipid resonances: (**1**; 0.90 ppm C**H**3CH2CH2C=C, **2**; 1.16 ppm CH3CH2C**H**2, **3**; 1.33 ppm C**H**2CH2CH2CO in fatty acyls, **4**; 1.37, 1.41 ppm C**H**2CH2CO, **5** 1.59 ppm C**H**2CH2C=C, **6**: 2.02 ppm C**H**2CH2CH2CH3, **7**; 2.24 ppm C**H**2C=C, **8**; 2.80 ppm C=CC**H**2C=C, **9**; 5.33 unsaturated lipids C=C**H**CH2CH2). Other: Acetate (**Ac**) 1.90 ppm (singlet); Succinate (**Suc**) at 2.29 ppm (singlet); small signal from **TMAO** at 3.27ppm.

Results– Fig. 2 shows representative 1D NMR spectra from living *C. elegans*. Most resonances are assigned to lipids. Additional resonances such as acetate and succinate are depicted. Spectra from 2D TOBSY HRMAS acquired as described in (4) exhibited additional resonances (not shown here).

Discussion– This work opens up perspectives for the use of ^{1}H HRMAS-NMR as a molecular profiling approach for small model organisms such as *C. elegans*. Because it is amenable to high throughput screening, functional genomics including RNAi, genetic screening, and has been used to study processes such as aging, fat storage, cell death, oxidative stress and complex metabolic behaviors, this approach may lead to a functional and integrated metabolomic mapping of the *C. elegans* genome at the systems biology level.

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