Supporting information

Simultaneous Assessment of Intracellular and Extracellular pH using Hyperpolarized [1-^{13}C]Alanine Ethyl Ester

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Synthesis of [1-^{13}C]alanine ethyl ester

[1-^{13}C]-Alanine (0.2 g, 2.22 mmol) was dissolved in 3.0 mL of dry ethanol. Dry HCl gas (obtained by dropping conc. H_2SO_4 to NaCl) was bubbled through the solution for 3 h with stirring. The mixture was refluxed for 12 h under inert condition. The solvent was removed under reduced pressure to yield the product, and the product obtained was crystallization using methanol/diethyl ether to afford white solid. Yield 85%.[1] ^{1}H-NMR (CD_3OD, 400 MHz) δ (ppm): 1.32 (t, 3H, J = 7.2 Hz), 1.55 (dd, 3H, J_{H-H} = 6.5 Hz, J_{C-H} = 4.0 Hz), 4.09 (m, 1H), 4.29 (m, 2H); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm): 14.35, 16.21, 50.16, 63.58, 170.94; ESI-MS: *m/z*: calcd for $[M+H]^+$: 119.0902; found 119.0863.

Dynamic nuclear polarization

[1-^{13}C]-L-alanine samples were prepared as described previously.[2] 6.2-M [1-^{13}C]-L-alanine ethyl ester samples were prepared by mixing with 15-mM OX063 in 1:3 gylcerol:water mixture. For polarization, [1-^{13}C]-L-alanine (75 μL) or [1-^{13}C]-L-alanine ethyl ester sample (75 μL) was inserted into a sample vial, assembled to the rest of the research fluid path (GE Healthcare, Waukesha MI, USA), and polarized using a SPINlab™ DNP polarizer (GE Healthcare) that operates at ~0.8 K in 5 T. The optimal microwave frequency and power calibrated for [1-^{13}C]pyruvate were used to irradiate the samples. 16-mL dissolution media containing 0.1 g/L of disodium ethylenediaminetetraacetate (Na_2EDTA) was prepared in the dissolution syringe. Each sample was polarized for approximately 5 h. Immediately after dissolution, HP [1-^{13}C]-L-alanine ethyl ester solution was mixed with 1 mL of Tris-HCl buffer at pH 7.4 for a final dissolution of 80-mM of alanine ethyl ester at pH 7.4. HP [1-^{13}C]-L-alanine solution, 250 mL of a buffer solution (a mixture of 6.1 mL of 121-mM HCl, 100 mg/L of disodium EDTA, 40-mM sodium phosphate buffer) was used.[2]

T_1 and polarization measurement

In vitro T_1 and liquid-state polarization level of [1-^{13}C]-L-alanine and [1-^{13}C]-L-alanine ethyl ester were measured using a clinical 3 T 750w Discovery wide-bore MRI scanner (GE Healthcare). pH-neutralized 5 mL of the HP solution was collected in a 6-mL syringe and placed at the center of a transmit/receive ^{1}H/^{13}C dual-tuned birdcage rat coil (GE Healthcare, inner diameter = 80 mm) in

the scanner, immediately followed by a non-selective data acquisition of ^{13}C free induction decay (FID) every 3 s (flip angle = 5.625°, pulse width = 24 μs, spectral width = 5,000 Hz, spectral point = 2,048). The collected data was apodized, zero-fitted, and applied by a fast Fourier transform (FFT) using MATLAB (Mathworks, Natick, MA, USA). Alanine or alanine ethyl ester peak was integrated at each timepoint, corrected by the RF losses, and the dynamic decay was fitted to a mono-exponential decay to estimate the T_1. Corresponding thermal polarization was also measured from the solution after adding 5-mM gadolinium using a non-selective FID sequence (flip angle = 90°, pulse width = 264 μs, number of averages = 600, TR = 10 s). The liquid-state polarization level at the time of dissolution was calculated by extrapolating the dynamic decay of each HP peak by the recorded dissolution-to-scan time (20 – 30 s).

HP phantom experiments

pH-sensing capability of [1-^{13}C]-L-alanine ethyl ester was evaluated using Eppendorf tubes, containing 200-μL of Tris-HCl buffer at pH 6.5, 7.0 and 7.5, respectively. HP [1-^{13}C]-L-alanine ethyl ester was inserted into the tubes (0.8 mL per tube) and mixed with the buffer solutions prior to placing at the center of the rat coil. To minimize the B_0 inhomogeneity, optimal shim currents were pre-calculated by a ^{1}H point-resolved spectroscopy (PRESS) sequence using an identical set of Eppendorf tubes filled with 1-mL water. A single timepoint two-dimensional free-induction decay (FID) chemical shift imaging (CSI) was acquired in the coronal plane (field of view = 80 mm × 80 mm, matrix size = 12 × 12, slice thickness = 2 cm, spectral width = 5,000 Hz, #spectral point = 256, repetition time = 75 ms, flip angle = 10°). The acquired data was reconstructed as previously described using MATLAB.[3]

Animal preparation and *in vivo* experiments

Healthy male Wistar rats (body weight = 220 to 587 g, n = 7, some animals were used in both studies) were anesthetized with 2 – 3 % isoflurane in oxygen (~1.5 L/min) with tail vein catheter were put at the center of the MR bore. Prior to HP ^{13}C injections, a three-plane ^{1}H gradient images acquired using the body coil for localization (field of view = 12 cm × 12 cm, slice thickness = 5 mm, flip-angle = 30°, echo time = 1.9 ms, repetition time = 6.1 ms). For HP ^{13}C, RF excitation and signal acquisition were obtained through custom-built ^{13}C surface coil (single loop, diameter = 28

mm) over the liver. A dose of 1 mmol/kg body weight of [1-^{13}C]-L-alanine or [1-^{13}C]-L-alanine ethyl ester solution was administered as a bolus 15 s after the dissolution through the tail vein catheter at a rate of 0.25 mL/s, immediately followed by a dynamic ^{13}C MRS scan (FID CSI, 10° hard pulse RF excitation, repetition time = 3 s, scan time = 4 min). Each animal was imaged twice with at least a 30-min time interval between the injections: one with HP alanine and the other with HP alanine ethyl ester. The concentration of HP [1-^{13}C]-L-alanine ethyl ester was 80-mM and the HP [1-^{13}C]-L-alanine concentration was kept the same to facilitate the performance comparison between alanine and alanine ethyl ester. Animal vital signs were monitored throughout the experiments. Respiratory rate was maintained at 50 – 60 breaths/min. All procedures were approved by the local Institutional Animal Care and Use Committee. The collected data was reconstructed as previously described.[3]

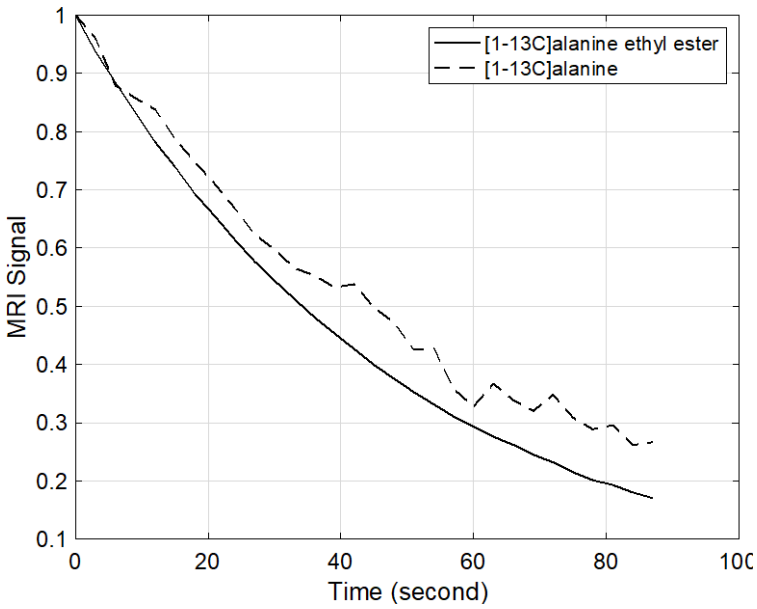

Figure S1. T_1 decay of liquid-state HP [1-^{13}C]-L-alanine ethyl ester and [1-^{13}C]-L-alanine.

Decay curve representation of Figure 1B.

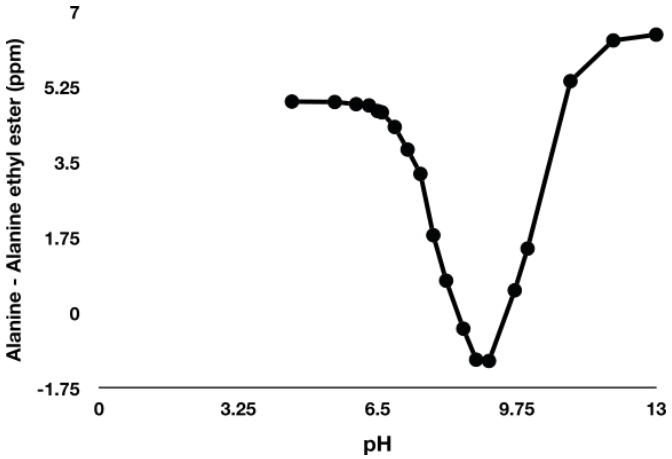

Figure S2. pH *vs* difference of [1-^{13}C]alanine ethyl ester and [1-^{13}C]alanine chemical shifts.

Difference of chemical shifts between [1-^{13}C]-L-alanine and [1-^{13}C]-L-alanine ethyl ester in aqueous solution with pH ranging from 4.5 to 13.0.

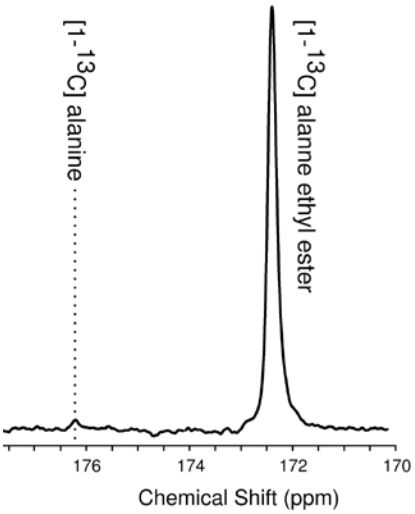

Figure S3. Alanine ethyl ester degradation in blood.

NMR spectrum of 1 mL of fresh rat blood serum in 2 mL of 10-mM [1-^{13}C]-L-alanine ethyl ester after incubation for 4 minutes (9.4 T). [1-^{13}C]-L-alanine ethyl ester (172.3 ppm), [1-^{13}C]-L-alanine (176.3 ppm).

pH	Alanine		Alanine ethyl ester	
4.5	176.62	176.61	171.72	171.69
5.5	176.66	176.62	171.76	171.72
6	176.62	176.61	171.78	171.75
6.3	176.67	176.61	171.83	171.81
6.5	176.63	176.61	171.96	171.9
6.6	176.64	176.62	172.01	171.93
6.9	176.66	176.61	172.32	172.31
7.2	176.66	176.63	172.86	172.84
7.5	176.67	176.66	173.44	173.43
7.8	176.64	176.88	174.98	174.93
8.1	176.99	176.91	176.22	176.18
8.5	177.00	176.95	177.35	177.33
8.8	177.3	177.36	178.44	178.39
9.1	177.85	177.81	178.95	178.94
9.3			179.28	179.28
9.4	178.44	179.2		
9.7	180.07	180	179.51	179.51
10	181.01	181.06	179.55	179.53
10.5			179.61	179.62
10.6	183.97	184.02		
11	185.08	185	179.66	179.65
11.5			179.66	179.66
12	185.98	185.99	179.65	179.66
13	186.13	186.12	179.66	179.66

Table S1. pH-dependence of chemical shifts of [1-^{13}C]-L-alanine and [1-^{13}C]-L-alanine ethyl ester.

Chemical shifts of [1-^{13}C]-L-alanine and [1-^{13}C]-L-alanine ethyl ester in aqueous solution with pH ranging from 4.5 to 13.0 (9.4 T).

References

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(2) Park, J. M.; Khemtong, C.; Liu, S.-C.; Hurd, R. E.; Spielman, D. M. In vivo assessment of intracellular redox state in rat liver using hyperpolarized [1-(13) C]Alanine. *Magn Reson Med* **2017**, *77* (5), 1741–1748.

(3) Park, J. M.; Josan, S.; Grafendorfer, T.; Yen, Y.-F.; Hurd, R. E.; Spielman, D. M.; Mayer, D. Measuring mitochondrial metabolism in rat brain in vivo using MR Spectroscopy of hyperpolarized [2-^{13}C]pyruvate. *NMR Biomed* **2013**, *26* (10), 1197–1203.