Relayed carbon-13 magnetization transfer. Detection of malate dehydrogenase reaction in vivo

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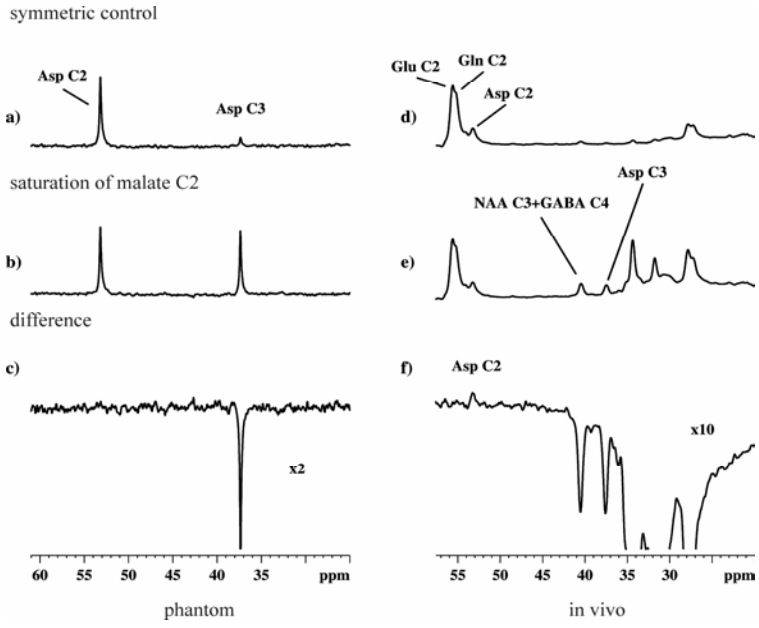
Introduction Malate dehydrogenase (MDH) catalyzes a fast and near equilibrium reaction in vivo: L-malate + NAD^+ ↔ oxaloacetate + NADH + H^+. The MDH reaction has been beyond the reach of in vivo MRS because of the low concentration of oxaloacetate (~0.005 μmol/g) and malate. Recently in vivo ^{13}C magnetization (saturation) transfer effect of the aspartate aminotransferase (AAT) reaction has been reported (1). Here we report relaying perturbation of magnetization between malate and aspartate via the rapidly turning over oxaloacetate, which is shared by both MDH and AAT reactions. Relayed ^{13}C magnetization transfer allows probing the MDH reaction in vivo by saturating the undetectable malate C2 at 71.2 ppm and detecting changes in the MRS-detectable aspartate C2 signal at 53.2 ppm.

Theory Using modified Bloch-McConnell equations for the longitudinal magnetization of malate, oxaloacetate and aspartate C2 carbons it can be shown that the relative change in aspartate C2 signal intensity ($[\Delta A]/[A]_0$) due to steady state saturation of malate C2 is related to the flux rate of the MDH reaction (V_{MDH}) by $[\Delta A]/[A]_0 = k_{AO}/(k_{AO} + T_{1A}^{-1}(1 + V_{AAT}/V_{MDH}))$ [1], where V_{AAT} and k_{AO} are rate and pseudo first order rate constant of the aspartate → oxaloacetate flux catalyzed by AAT and T_{1A} is T_1 of aspartate C2 in the absence of chemical exchange.

Experimental Methods The experimental methods were similar to those used in ref (1) for determination of V_{AAT}. Briefly, all experiments were performed using a Bruker 11.7 Tesla spectrometer interfaced to an 89-mm bore vertical magnet. An in-house transmit/receive concentric surface ^{13}C RF coil system was used. Male Sprague-Dawley rats (167-186 g, n = 8) fasted for 24 hours were studied to measure the relayed magnetization transfer effect (n = 5) and T_{1A} (n = 3) in the rat brain. The rats were orally intubated and ventilated with a mixture of 70% N_2O/30% O_2 and 1.5% isoflurane. Intravenous infusion of [1,6-$^{13}C_2$]glucose was started approximately 1 hr prior to in vivo ^{13}C data acquisition. 90° excitation, surface-coil-localized acquisition was used to measure the relayed magnetization transfer effect. TR = 7.6 sec. When the relayed ^{13}C magnetization transfer spectra were acquired, the malate C2 at 71.2 ppm was saturated using a train of spectrally selective 2-ms Gaussian pulses with a nominal flip angle of 180° spaced 12 ms apart. When the control spectra were acquired, the saturating pulse train was placed at an equal spectral distance from aspartate C2 at 53.2 ppm but on the opposite side of malate C2. The saturated and control spectra were interleaved every FID.

Results The magnetization transfer pulse sequence was first tested on a phantom (100 mM aspartate, pH = 7.0; 37 °C) as shown in Figs. a-c. LB = 15 Hz. In Fig a, the Gaussian pulse train was placed at 35.2 ppm (control frequency), which partially saturated aspartate C3 at 37.4 ppm. In Fig. b, the Gaussian pulse train was placed at the malate C2 frequency (71.2 ppm). In the difference spectrum shown in Fig c, complete cancellation of aspartate C2 was obtained. Figs. d-f show the in vivo results of saturating malate C2. LB = 30 Hz. NS = 2688 (summed from two rats). In Fig. d, the Gaussian pulse train was placed at 35.2 ppm. Glutamate C2, glutamine C2, aspartate C2, GABA C4 + N-acetylaspartate C3, aspartate C3, glutamate C4, glutamine C4, glutamate C3, glutamine C3 were observed as expected. Fig. e shows the in vivo spectrum with the Gaussian pulse train placed at 71.2 ppm. The difference spectrum shown in Fig. f contains a small but consistent and conspicuous signal at the resonance frequency of aspartate C2. In comparison, the nearby much more intense glutamate and glutamine C2 resonances were cancelled in Fig f. $[\Delta A]/[A]_0$ was determined to be 0.08 ± 0.01 (mean ± SD, n = 5, NS = 1152-1536 per rat). Note that the negative signals upfield of aspartate C2 in Fig f were due to saturation of the N-acetylaspartate C3, GABA C4, glutamate C4, glutamine C4, glutamate C3 and glutamine C3 resonances by the Gaussian 180° pulse placed at the control frequency. T_{1A} was determined to be 2.2 ± 0.1 sec (mean ± SD, n = 3, NS = 1536-2560 per rat). Using the data from ref (1), k_{AO} and V_{AAT} (for the oxaloacetate ↔ aspartate half reaction) were calculated to be 0.17 ± 0.02 sec^{-1} and 29 ± 4 μmol/g wet weight/min, respectively. From eq. [1], V_{MDH} was calculated to be 9 ± 2 μmol/g wet weight/min (mean ± SD, n = 5), corresponding to a $t_{1/2}$ time of approximately 1.6 sec for malate.



Discussion Any leakage of perturbed magnetization of oxaloacetate or influx of unperturbed magnetization into oxaloacetate causes an underestimation of V_{MDH}. Fortunately, compared to the fast reactions catalyzed by MDH and AAT, all other reactions involving oxaloacetate (e.g., that catalyzed by pyruvate carboxylase, citrate synthetase, or oxaloacetate decarboxylase) are negligibly slow in brain based on previous enzymatic studies. Previous in vitro findings obtained from several species suggest that MDH is operating in vivo at well below its K_m values for oxaloacetate and NADH (2). In this study, in vivo V_{MDH} was found to be ~13% of its V_{max} determined from homogenate of rat cerebral cortex (3), providing evidence for the low availability of substrates for this enzyme in vivo. The relatively large frequency separation among aspartate, oxaloacetate and malate C2 resonances has made it possible to completely eliminate any RF spillover effect by the use of a spectrally selective Gaussian pulse train for saturating malate C2 (Fig. c). Note that malate C2 is coupled to malate H2 with $^1J_{CH}$ = 146 Hz. The bandwidth of the Gaussian pulse has to be significantly greater than $^1J_{CH}$, preventing the use of very selective RF pulses. In conclusion, it is demonstrated that the rapid MDH reaction is detectable in vivo using relayed ^{13}C magnetization transfer at 11.7 Tesla. The relay strategy proposed here may be extended to detecting other enzyme-catalyzed reactions and therefore providing valuable insight into the catalytic action of enzymes both in vivo and in vitro.

References

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