Available online at www.sciencedirect.com
ScienceDirect
Journal of Magnetic Resonance 184 (2007) 344–349
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Communication

Relayed ^{13}C magnetization transfer: Detection of malate dehydrogenase reaction in vivo

Jehoon Yang, Jun Shen *

Molecular Imaging Branch, National Institute of Mental Health Intramural Research Program, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892-1527, USA

Received 1 September 2006; revised 3 November 2006
Available online 27 November 2006

Abstract

Malate dehydrogenase catalyzes rapid interconversion between dilute metabolites oxaloacetate and malate. Both oxaloacetate and malate are below the detection threshold of in vivo MRS. Oxaloacetate is also in rapid exchange with aspartate catalyzed by aspartate aminotransferase, the latter metabolite is observable in vivo using ^{13}C MRS. We hypothesized that the rapid turnover of oxaloacetate can effectively relay perturbation of magnetization between malate and aspartate. Here, we report indirect observation of the malate dehydrogenase reaction by saturating malate C2 resonance at 71.2 ppm and detecting a reduced aspartate C2 signal at 53.2 ppm due to relayed magnetization transfer via oxaloacetate C2 at 201.3 ppm. Using this strategy the rate of the cerebral malate dehydrogenase reaction was determined to be 9 ± 2 μmol/g wet weight/min (means ± SD, $n = 5$) at 11.7 Tesla in anesthetized adult rats infused with [1,6-$^{13}C_2$]glucose.
Published by Elsevier Inc.

Keywords: In vivo MRS; Magnetization transfer; Malate dehydrogenase; Carbon-13; Enzymology

1. Introduction

Malate dehydrogenase (MDH; L-malate:NAD oxidoreductase; EC 1.1.1.37) catalyzes the reversible interconversion between L-malate and oxaloacetate using nicotinamide adenine dinucleotide (NAD) as a coenzyme

$$\text{L} - \text{malate} + \text{NAD}^+ \leftrightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+$$

The equilibrium of the MDH reaction energetically favors the reduction of oxaloacetate into malate. MDH is found in all eukaryotic cells as two isozymes: mitochondrial and cytoplasmic. The mitochondrial MDH is the final enzyme of the tricarboxylic acid (TCA) cycle. It oxidizes malate into oxaloacetate. Both mitochondrial and cytoplasmic MDH participate in the malate–aspartate shuttle which carries the reducing equivalents from the cytoplasm into the mitochondria for oxidation. In cytoplasm, MDH plays an important role in generating NADPH needed for reductive biosynthesis. The carbon skeleton of malate transported out of mitochondria is also utilized for gluconeogenesis.

Previously, applications of enzyme-specific in vivo magnetization (saturation or inversion) transfer spectroscopy were limited to ^{31}P MRS studies of creatine kinase and ATP exchange reactions [1,2]. Very recently, we discovered the in vivo magnetization transfer effect of aspartate aminotransferase (AAT) reaction. It was shown that the rapid and reversible AAT half-reactions (glutamate ↔ α-ketoglutarate and aspartate ↔ oxaloacetate) are detectable in vivo using ^{13}C magnetization transfer (CMT) by saturating the carbonyl carbon (C2) of either α-ketoglutarate at 206.0 ppm or oxaloacetate at 201.3 ppm [3]. The CMT effect of the lactate dehydrogenase (LDH) reaction (pyruvate + NADH + H^+ ↔ lactate + NAD^+) was similarly detected in vivo in bicuculline-treated rats and in glioma by saturating the pyruvate C2 resonance at 207.9 ppm [4]. Like AAT and LDH, MDH also catalyzes a near equilibrium reaction in vivo [5]. In the homogenate of adult

* Corresponding author. Fax: +1 301 480 2397.
E-mail address: shenj@intra.nimh.nih.gov (J. Shen).

1090-7807/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.jmr.2006.11.002

rat cerebral cortex, the activity of MDH (V_{max}) was determined to be 69 μmol/g wet weight/min [6]. Because of the extremely low in vivo concentration of oxaloacetate (approximately 0.005 μmol/g wet weight in rat brain, [5]) and the rapid AAT- and MDH-catalyzed reactions, there exists an interesting possibility of relaying perturbation of magnetization between malate and aspartate via the rapidly turning over oxaloacetate, which is shared by both reactions.

Altered MDH activity in the abnormal brain has been reported using postmortem analytical methods (e.g., [7]). The antipsychotic drug haloperidol has also been found to elevate the transcript-encoding of cerebral cytoplasmic MDH [8]. Due to the low in vivo concentration of malate (approximately 0.3–0.4 μmol/g wet weight in brain [5,9]), malate and the MDH reaction are generally considered to be beyond the capability of in vivo MRS. With the rapidly increasing number of high field magnets available for human and animal studies, it is desirable to further extend the scope of the "signal-hungry" in vivo MRS to include previously undetectable signals. Relayed ^{13}C magnetization transfer could offer a mean to probe the malate dehydrogenase 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. The successful in vivo demonstration of this strategy in isoflurane-anesthetized adult rat brain using an 11.7 Tesla scanner is presented here. The standard steady state saturation transfer method, which produces the maximum SNR, was employed to measure the rate of the MDH reaction. To the best of our knowledge, no in vivo measurement of the malate dehydrogenase-catalyzed exchange rate had been reported prior to the current work.

2. Theory

The following pseudo first order rate constants are defined: k_{MO} for malate → oxaloacetate; k_{OM} for oxaloacetate → malate; k_{OA} for oxaloacetate → aspartate; and k_{AO} for aspartate → oxaloacetate. The modified Bloch–McConnell equations for the longitudinal magnetization of malate, oxaloacetate and aspartate C2 carbons ([M], [O] and [A], respectively) are

$$d[M]/dt = ([M]_0[M])/T_{1M} + k_{OM}[O] - k_{MO}[M] \quad (1)$$

$$d[O]/dt = ([O]_0[O])/T_{1O} + k_{MO}[M] + k_{AO}[A] - (k_{OM} + k_{OA})[O] \quad (2)$$

$$d[A]/dt = ([A]_0[A])/T_{1A} + k_{OA}[O] - k_{AO}[A] \quad (3)$$

where T_{1M}, T_{1O} and T_{1A} are the spin–lattice relaxation times of malate, oxaloacetate and aspartate C2 carbons, respectively, in the absence of any chemical exchange, and $[M]_0$, $[O]_0$ and $[A]_0$ are the thermal equilibrium values of [M], [O] and [A], respectively. We further define that $V_{AAT} \equiv k_{AO}[A]_0 = k_{OA}[O]_0$ and $V_{MDH} \equiv k_{MO}[M]_0 = k_{OM}[O]_0$.

Eqs. (2) and (3) can be solved for the steady state condition $d[O]/dt = d[A]/dt = 0$ and $[M] = 0$ resulting from RF saturation of the malate C2 resonance at 71.2 ppm

$$[O]_{ss} = ([O]_0/T_{1O} + k_{AO}[A])/(T_{1O}^{-1} + k_{OM} + k_{OA}) \quad (4)$$

$$[A]_{ss}/[A]_0 = (T_{1A}^{-1} + \alpha)/(T_{1A}^{-1} + k_{AO} + \beta) \quad (5)$$

where $[O]_{ss}$ and $[A]_{ss}$ are the steady state magnetization of oxaloacetate and aspartate C2 resonances and

$$\alpha = k_{AO}/(1 + T_{1O}(k_{OM} + k_{OA})) \quad (6)$$

$$\beta = k_{OA}k_{AO}/(T_{1O}^{-1} + k_{OM} + k_{OA}) \quad (7)$$

The relationship $k_{AO}[A]_0 = k_{OA}[O]_0$ was used in deriving Eq. (5). Note that if $\alpha = \beta = 0$ Eq. (5) reduces to the well-known saturation transfer equation for two-site exchange ($[A]_{ss}/[A]_0 = T_{1A}^{-1}/(T_{1A}^{-1} + k_{AO})$, same as Eq. (11) in Ref. [2]).

Since $[O]_0 \ll ([M]_0, [A]_0)$, the turnover rate of oxaloacetate is expected to be several orders of magnitude greater than the longitudinal relaxation rate of its unprotonated carbonyl carbon. That is, $k_{OA} \gg T_{1O}^{-1}$. Therefore, Eq. (5) can be rewritten into

$$[\Delta A]/[A]_0 = k_{AO}/(k_{AO} + T_{1A}^{-1}(1 + V_{AAT}/V_{MDH})) \quad (8)$$

where $[\Delta A] \equiv [A]_0 - [A]_{ss}$. The relationship $k_{OA}/k_{OM} = V_{AAT}/V_{MDH}$ was also used in deriving Eq. (8). Since $k_{AO}[A] \gg [O]_0/T_{1O}$ and $k_{OM} + k_{OA} \gg T_{1O}^{-1}$, it can be shown from Eq. (4) that

$$[O]_{ss}/[O]_0 = ([A]_{ss}/[A]_0)/(1 + V_{MDH}/V_{AAT}). \quad (9)$$

3. Experimental methods

The experimental methods were similar to those used in our previous publications [3,4]. Briefly, all experiments were performed using a Bruker 11.7 Tesla spectrometer interfaced to an 89-mm bore vertical magnet and ParaVision 3.0.1 (Bruker Biospin, Billerica, MA). An in-house transmit/receive concentric surface ^{13}C (circular, diameter: 10 mm)/^{1}H (octagonal, diagonal: 25 mm) RF coil system was used which is integrated to an animal handling system [10]. Male Sprague–Dawley rats (167–186 g, $n = 8$) fasted for 24 h with free access to drinking water were studied to measure the relayed CMT effect ($n = 5$) and the T_1 relaxation rate of aspartate C2 resonance ($n = 3$) in the rat brain as approved by the National Institute of Mental Health (NIMH) Animal Care and Use Committee. The rats were orally intubated and ventilated with a mixture of 70% N_2O/30% O_2 and 1.5% isoflurane. The left femoral artery was cannulated for measuring arterial blood gases (pO_2, pCO_2), pH, plasma glucose concentration using a blood analyzer (Bayer Rapidlab 860, East Walpole, MA), and for monitoring arterial blood pressure. One femoral vein was also cannulated for intravenous infusion of [1,6-$^{13}C_2$]glucose (1-^{13}C, fractional enrichment: 0.99; 6-^{13}C, fractional enrichment: 0.97, Cambridge Isotope Laboratories, Andover, MA). The scalp was removed to minimize extracranial contamination of in vivo ^{13}C data. Intravenous infusion of [1,6-$^{13}C_2$]glucose was started approximately 1 h prior to in vivo ^{13}C data acquisition.