Fast ^{13}C Label Exchange between Mitochondria and Cytosol in Brain Revealed by Saturation Transfer Spectroscopy

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Introduction

MRS has been used to follow the flow of ^{13}C labels from enriched substrates, via TCA cycle in mitochondria, to many metabolites including brain glutamate and aspartate. MRS detects the total, and therefore predominantly cytosolic, signals while the mitochondrial pools are negligibly small. Whether ^{13}C label exchange between mitochondria and cytosol (V_x) is slow ($V_x \approx V_{TCA}$) or fast ($V_x >> V_{TCA}$) in brain is highly controversial (1). If $V_x \approx V_{TCA}$, V_{TCA} will depend on V_x. In brain, many enzymes from the TCA cycle are found in the cytosol of neurons and astroglia. But others (e.g., fumarase) are exclusively localized to mitochondria (1). Fumarase (FA), malate dehydrogenase (MDH), and aspartate aminotransferase (AAT) catalyze a linear chain of rapid, near equilibrium exchange reactions in mitochondria (2). We hypothesized that a fast V_x should cause a detectable ^{13}C saturation transfer effect on cytosolic aspartate when fumarate is irradiated using radiofrequency pulses.

Materials and Methods

Fig. 1 shows the exchange between cytosol and mitochondria on the four-carbon side of the TCA cycle. Cytosolic malate enters mitochondria via OGC (the malate/α-ketoglutarate exchanger). Aspartate leaves mitochondria via AGC (the aspartate/glutamate carrier) (2). The rapid exchanges between malate and oxalocacetate and between oxaloacetate and aspartate are catalyzed by MDH and AAT, respectively. In mitochondria, malate also rapidly exchanges with fumarate catalyzed by FA. In Fig. 1, only cytosolic aspartate is detectable by MRS in vivo. To estimate V_x using ^{13}C saturation transfer the rapidly exchanging small mitochondrial fumarate, malate, oxaloacetate, and aspartate pools can be lumped into a single mitochondrial site, which is saturated by radiofrequency irradiation of fumarate C2 at 136.1 ppm. This simplified procedure attributes the loss of saturation inside mitochondria to V_x. V_x calculated from this simplification therefore represents its lower limit. From the literature (2-4), cytosolic [aspartate] = 2.8 μmol/g, [malate] = 0.3 μmol/g, $T_{1aspartate}$ = 2.2 sec. Cytosolic oxaloaceate ↔ aspartate rate (V_{AAT}) = 29 μmol/g/min, malate ↔ oxaloaceate (V_{MDH}) = 9 μmol/g/min. From the Bloch-McConnell equations $V_x = (f_{oxaloacetate}/f_{malate} - 1)V_{MDH} + (1/f_{malate} - 1)[malate]/T_{1malate}$, where f denotes fraction of unsaturated magnetization, $f_{oxaloacetate} = (f_{aspartate}V_{AAT} - [aspartate](1 - f_{aspartate})/T_{1aspartate})/V_{AAT}$ and $f_{malate} = (f_{oxaloacetate}(V_{AAT} + V_{MDH}) - f_{aspartate}V_{AAT})/V_{MDH}$. Only $T_{1malate}$ is unavailable because malate is below the detection threshold of in vivo MRS. V_x can be calculated by assuming $T_{1malate} = 0.5\sim2.0 \times T_{1aspartate}$ because both malate and aspartate C2 carbons are singly protonated and the two molecules are small and similar in size (5).

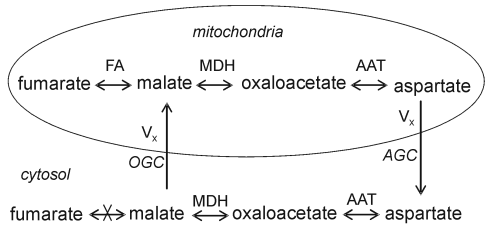

Fig. 1. The exchange of four-carbon molecules between cytosol and mitochondria (adapted from Fig. 46 in ref 2).

Isoflurane-anesthetized male Sprague-Dawley rats (176-213 g, n = 8) were intravenously infused with [1,6-$^{13}C_2$]glucose. Experiments were performed on a Bruker 11.7 Tesla spectrometer. The 90° excitation, surface-coil-localized, interleaved acquisition method (3) was used to measure ^{13}C saturation transfer between mitochondrial fumarate and cytosolic aspartate. When the ^{13}C magnetization transfer spectra were acquired, fumarate C2 at 136.1 ppm was saturated using a train of spectrally selective 2-ms Gaussian pulses with a nominal flip angle of 180° spaced 12 ms apart. The duration of the Gaussian pulse train was 7.3 sec. Data were zerofilled to 16 K and apodized using a matched filter for maximum sensitivity (lb = 30 Hz) prior to Fourier transformation.

Results and Discussion

Fig. 2 shows results summed from eight rats. In the difference spectrum (total NS = 20k), a small but well-defined peak at the resonance frequency of aspartate C2 (53.2 ppm) was clearly detected. In comparison, the nearby and much more intense α-glucose C6 (61.7 ppm), β-glucose C6 (61.8 ppm), glutamate C2 (55.2 ppm), glutamine C2 (55.1 ppm), and NAA C2 (54.0 ppm) resonances were completely cancelled. 1-$f_{aspartate}$ was determined to be 4.2%, with a relative standard deviation (rSD) of 15%. The rSD was estimated by integrating the aspartate C2 signal and its neighboring spectral regions in the difference spectrum using the same interval length. Using the Bloch-McConnell equations, V_x = 11~23 μmol/g/min for $T_{1malate} = 2.0\sim0.5 \times T_{1aspartate.}$ In saturation transfer it is the unidirectional flux from the observed spin to the saturated spin that is measured. For Fig. 1, the unidirectional cytosolic aspartate → cytosolic oxaloacetate → cytosolic malate → mitochondrial fumarate relay was measured. From the Bloch-McConnell equations, $f_{aspartate}(95.8\%) > f_{oxaloacetate}(84.7\%) > f_{malate}(49.1\%) > f_{fumarate}(0\%)$.

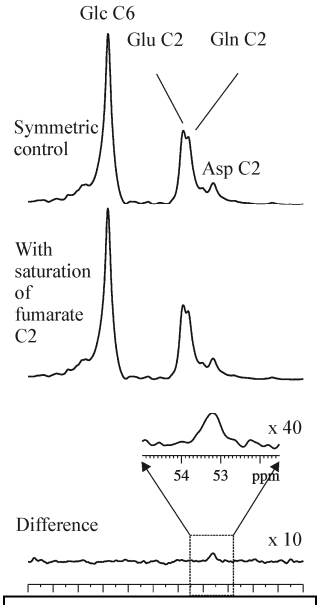

Fig. 2. In vivo ^{13}C saturation transfer results summed from eight rats.

Nearly all TCA intermediates are transported out of and into mitochondria through a host of exchangers and co-transporters (1,2). Carriers such as AGC and OGC couple the exchange of glutamate between mitochondria and cytosol on the five/six carbon side of the TCA cycle to that of aspartate on the four-carbon side (2). Under the isoflurane anesthesia of the current study, V_{TCA} = 0.40-0.48 μmol/g/min (6). The results here therefore clearly demonstrate that $V_x >> V_{TCA}$ regardless of $T_{1malate}$. The conclusion that ^{13}C label exchange between mitochondrial TCA cycle intermediates and cytosolic metabolites is rapid is independent of specific exchange models used for analyzing the ^{13}C magnetization transfer data; this is because V_x has to be fast enough on the time scale of ^{13}C T_1 relaxation to be detectable using saturation transfer. That is, for an exchange process to be detectable using saturation transfer, the exchange rate multiplied by T_1 of the observed signal has to be a significant fraction of the pool size of the observed signal. If $V_x \approx V_{TCA}$, $V_x \times T_{1aspartate} \approx 0.016$ μmol/g, corresponding to 1- $f_{aspartate}$ = 0.6% << the experimentally observed 4.2%.

References 1. Rodrigues et al, The Cerebral Tricarboxylic Acid Cycles, 2006; 2. Siesjo, Brain Energy Metabolism, 1978; 3. Shen, Magn Reson Med 54:1321, 2005; 4. Yang et al, J Magn Reson 184:344, 2007; 5. Wehrli, Topics in Carbon-13 NMR Spectroscopy, 1976; 6. Maekawa et al, Anesthesiology 65:144, 1986.