Slow Exchange Of α-Ketoglutarate/Glutamate Between Mitochondrial And Cytosolic Compartments Of Perfused Mouse Liver As Detected By ^{1}H *And* ^{2}H Decoupled ^{13}C NMR Spectroscopy.

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Abstract. The exchange by deuterium of the H2, H3*proR* and H3*proS* hydrogens of (2-^{13}C) glutamate was followed by proton *and* deuterium decoupled high resolution ^{13}C NMR (150.13MHz) of extracts prepared along mouse liver perfusions with (3-^{13}C) alanine in Krebs Ringer Bicarbonate containing 50% $^{2}H_2O$. H2 deuteration depicted two kinetic components, fast and slow, ocurring before or after H3*proS* deuteration. Similarly, fast and slow kinetic components could be resolved for H3*proS* deuteration, ocurring before or after H2 deuteration, respectively. These results reveal a slow exchange of α-ketoglutarate/glutamate between mitochondrial and cytosolic compartments in the ^{2}H-^{1}H exchange timescale.

Introduction. Dynamic ^{13}C NMR experiments monitor metabolic turnover as reflected in the kinetics of exchange of the naturally occurring ^{12}C atoms of metabolites by ^{13}C atoms derived from a ^{13}C enriched precursor. However, most carbon atoms of metabolites contain vicinaly or geminaly attached hydrogens, a circumstance which lead us to propose the possibility to monitor hydrogen turnover by ^{13}C NMR (1,2). Here we report a high resolution ^{13}C NMR study of the kinetics of exchange by deuterium of the H2, H3 and H3´ hydrogens of (2-^{13}C) glutamate. The turnover of some of these hydrogens occurred in some instances significantly faster than previously reported time courses of ^{13}C enrichment, allowing to follow the traffic of (2-^{13}C) glutamate through mitochondria and cytosol.

Materials and Methods. *Perfusion techniques and experimental design* - Mouse livers (ca 2,5 g) from 24h starved Swiss albino mice (ca 30g) were perfused for 30 minutes with Krebs Ringer Bicarbonate (KRB) buffer containing 6 mM (3-^{13}C) alanine in KRB under recirculating conditions (1). After this equilibration period the perfusion medium was changed to 6 mM (3-^{13}C) alanine in KRB containing 50% v.v $^{2}H_2O$ maintaining the recirculation for one, three, five, seven, ten, fifteen or thirty minutes. Perchloric acid extracts from these livers were prepared, neutralized with KOH, lyophilized and resuspended in $^{2}H_2O$ (99,9% ^{2}H) prior to high resolution ^{13}C NMR analysis.

High resolution ^{13}C NMR - ^{1}H decoupled *and* ^{2}H decoupled ^{13}C NMR spectra were obtained at 11.9 Tesla with a Bruker AVANCE 500 NMR spectrometer equipped with a commercial crioprobe refrigerated with liquid nitrogen. Conditions were: π/3 pulses, 25.06 KHz, 64K data table, 6s total cycle time and 4096 scans. ^{2}H decoupling was performed simultaneously with ^{1}H decoupling only during the acquisition, using the lock channel and a software driven lock switch. Isotopically shifted and unshifted resonances were quantified using the Windaisy and WinNMR programs.

Results. Fig. 1 shows a representative comparison of ^{1}H decoupled and ^{1}H *and* ^{2}H decoupled ^{13}C NMR spectra of the C2 resonance from (2-^{13}C) glutamate. Simultaneous ^{1}H *and* ^{2}H decoupling improved significantly both, the resolution and the sensitivity for the detection of the shifted an unshifted ^{2}H-^{13}C resonances (s,ss,dss,t,st in Fig 1). Similar experiments to those of Fig. 1, performed at increasing perfusion times, allowed to determine the kinetics of deuteration of the H2 and H3 hydrogens of (2-^{13}C) glutamate (Fig. 2).

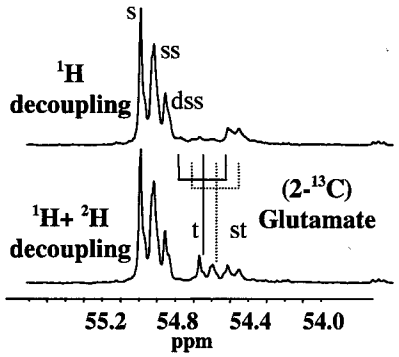

Fig. 1. ^{13}C NMR spectra of the C2 resonance from (2-^{13}C) glutamate. s: singlet= (2-^{13}C) glutamate, ss: shifted singet= (2-^{13}C, 3-^{2}H) glutamate, dss: doubly shifted singlet= (2-^{13}C, 3,3´-$^{2}H_2$) glutamate, t: triplet= (2-^{13}C, 2-^{2}H) glutamate, st: shifted triplet= (2-^{13}C, 3-^{2}H, 2-^{2}H) glutamate.

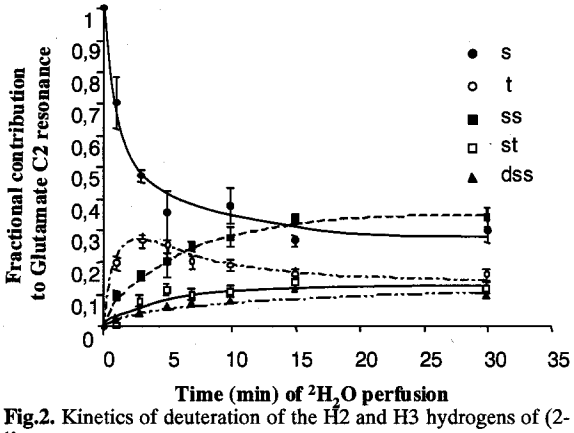

Fig.2. Kinetics of deuteration of the H2 and H3 hydrogens of (2-^{13}C) glutamate.

Discussion. The results obtained reveal that the H2 hydrogen is exchanged with two different kinetic components; one fast (t), corresponding to (2-^{13}C, 2-^{2}H) glutamate and another slow (st) corresponding to (2-^{13}C, 2-^{2}H, 3-^{2}H) glutamate. Similarly, the H3 hydrogen also depicts two kinetic components; fast (ss) corresponding to (2-^{13}C, 3-^{2}H) glutamate and slow (dss) corresponding to (2-^{13}C, 3,3´-$^{2}H_2$) glutamate. Fast and complete intracellular equilibration of (2-^{13}C) α-ketoglutarate/glutamate would result in single kinetics of H2 and H3 deuteration. This is not observed, suggesting that a kinetic limitation precludes the complete intracellular equilibration of α-ketoglutarate/glutamate. Possible processes accounting for this include mitochondrial transport and tricarboxylic acid cycle metabolism.

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Bibliography.

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