Kinetic Data From Cellular Assay Using Hyperpolarized ^{13}C-DNP-NMR

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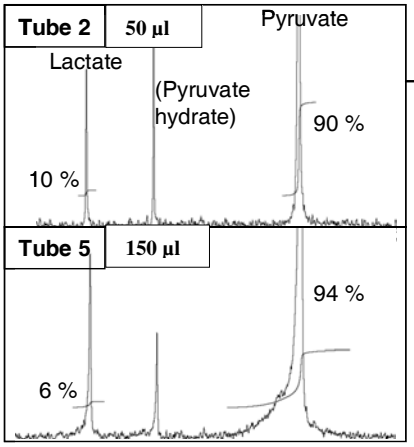
Introduction: *In vivo* screening of potential diagnostic markers with Dynamic Nuclear Polarization (DNP) NMR is tedious and time-consuming. When studying metabolism with the DNP-NMR technique, substrate concentrations are usually higher than in normal blood and cellular concentrations, and the time window is far away from steady state conditions. It is therefore not likely that much insight can be gained from kinetics reported in the literature. In addition, although both uptake and conversion rates and K_M values are reported for many of the enzymes involved in the studied metabolism, it can be envisioned complicated to model all the enzymatic processes involved in getting from the substrate to product, in particular in specific species or tissues not previously characterized. We have therefore developed an *in vitro* cellular assay, which allows determination of valuable kinetic data from a single-shot NMR acquisition. The full process of substrate transport into the cells followed by conversion to the product is measured and quantified to K_M and v_{max} for the whole system (K_{Msys} and v_{maxsys}) using a well studied substrate for DNP-NMR, $^{13}C_1$-Pyruvate [1,2]. The assay is fast, easy to handle and mimics the conditions for *in vivo* ^{13}C-DNP experiments.

Methods: $^{13}C_1$-pyruvate was hyperpolarized using DNP and dissolved following a published standard procedure for producing hyperpolarized substrates [3]. One hyperpolarized sample was used to perform 5 cell experiments in random order where 10-150 µl of 13.5 mM hyperpolarized $^{13}C_1$-pyruvate was mixed into 500 µl cell suspension ($10 \cdot 10^6$ breast carcinoma (MDA) cells). The fraction lactate compared to the total lactate and pyruvate integral was calculated from single ^{13}C-spectra acquired on a 400 MHz Varian system 34 s after mixing. Based on a Michaelis-Menten type analysis, the assay provides two constants, v_{maxsys} and K_{Msys}, which describe the overall kinetics of the metabolic system. This nomenclature refers to the total process of going from the substrate to the product. v_{maxsys} is therefore the maximum rate for the rate limiting enzyme in the reaction and K_{Msys} is the K_M for the rate limiting enzyme, Figure 1.



Sample volume [µl]	[S] (mM)	Fraction Lactate	[P] (mM)	v_0 (mM/s)
10	0.26	16	0.04	$1.2 \cdot 10^{-3}$
25	0.64	11	0.07	$2.1 \cdot 10^{-3}$
50	1.22	10	0.13	$3.7 \cdot 10^{-3}$
100	2.23	7	0.17	$4.9 \cdot 10^{-3}$
150	3.12	6	0.18	$5.2 \cdot 10^{-3}$

Cell line	v_{maxsys} (10^{-3} mM/s)	K_{Msys} (mM)
MDA	8.1 ± 0.64	1.6 ± 0.27

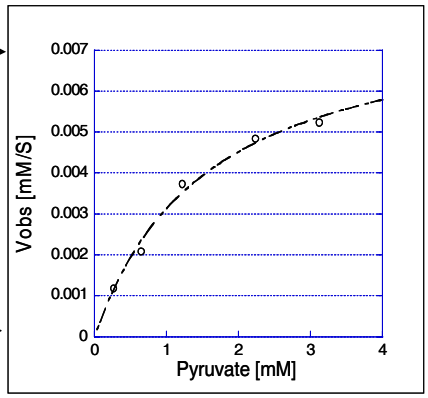


Figure 1. ^{13}C spectra from experiments 2 and 5 in the cell assay. Each spectrum was recorded 34 s after mixing hyperpolarized pyruvate with MDA cells (left). From each experiment, the fraction lactate is extracted and the initial rate, v_0, is calculated. Rates are fitted to Michaelis-Menten kinetics to determine parameters for v_{maxsys} and K_{Msys} (right).

Results and discussion: From a single DNP dissolution, global values for the kinetic parameters v_{max} and K_M can be obtained for a metabolic system. These values can be obtained for many different substrates and in different cell lines and provide important comparable information. In the relatively narrow concentration window (1-10 mM) and very short time window (1-2 min), under which the *in vivo* DNP-experiment is carried out, this fast and easy to handle cellular assay provides a good basis for the screening of potential diagnostic markers.

Acknowledgement: GE Healthcare is gratefully acknowledged for sponsoring the research.

References: [1] Golman *et al.*, PNAS, 25; 103(30):11270-5, 2006, [2] Golman *et al.*, Cancer Res 66(22), 2006, [3] Ardenkjær-Larsen *et al.*, PNAS 100:10158, 2003.