Hyperpolarized ^{13}C-DNP-NMR allow metabolic *in vitro* studies over minutes

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Introduction: An experimental setup is needed to evaluate and establish biological models for diagnostic markers with Dynamic Nuclear Polarization (DNP) NMR [1]. DNP-NMR applied to cell cultures provides a non-invasive mean to probe metabolism of the cells and is as such a good model for the *in vivo* situation. The model is versatile to different substrates, low in costs and fast compared to an *in vivo* model. It is performed at similar experimental conditions (time and concentration) [2,3]. It is strictly focused on characterizing the metabolic substrate/product relationship. The development of a cellular based DNP-NMR assay serves two different purposes; it provides a screening essay based on which a metabolic profile for a substrate can be obtained. The essay also provides a way to extract kinetic parameters for the metabolic system. With this essay individual design of substrates as diagnostic or therapeutic makers can be obtained.

Methods: 500 µl of a cell-suspension ($20 \cdot 10^6$ cells/ml) is added to a 5-mm NMR tube and placed in a water bath at 37ºC (Fig. 1). One harvest will provide for 5-6 sample tubes, depending on the exact confluence of the cells and the cell type. Approx. 70 µmol ^{13}C labelled substrate is polarized and subsequently dissolved in 6 ml solvent, yielding a concentration of approx. 15 mM. The pH of the dissolved sample is buffered to 6.5 - 8.5. The pH of the cell suspension is buffered to 7.4. The following procedure is followed in the cell experiment:

- The dissolved sample is collected in a 60 ml tube with a 5 ml syringe connected.
- A first volume of the dissolved sample is used for NMR-estimation of the polarization. 1 ml of the remaining sample is transferred to an Eppendorph tube which is thermostated to 37ºC.
- From the Eppendorph tube, an amount of sample is transferred with an automatic pipette on the inside top of the nmr tube (without contact to the cells)
    - The cell suspension is mixed with the substrate by turning of the 5-mm NMR tube with the cap on.
    - Time is monitored from when the tube is turned until a single-scan ^{13}C-NMR acquisition is triggered (4-5s)
    - NMR is triggered by manual coordination

This procedure is then repeated e.g. with addition of different amounts of substrate to the rest of the tubes. The total experimental time is 4-5 minutes depending on the number of tubes and the exact NMR protocol used in the experiment.

Figure 1. *The water-bath containing tubes with cell suspensions.*

Results and Discussion: The cellular DNP-NMR assay provides a basis for extraction of several types of information regarding the substrate/product relationship in a metabolic system. The concentration of the product formed can be determined from the ratio between integrated NMR peaks in a spectrum collected after a specific incubation time (e.g. 20 s, shown in Fig. 2a). This way, a series of single-scan ^{13}C-spectra can be collected with variations in e.g. substrate concentration from which kinetic data can be extracted. Alternatively, the product formation may be followed as a function of time in one sub-experiment using a number of small pulse angles (Fig. 2b). In the latter experiment, corrections for RF depolarization and T_1 relaxation are made before the kinetic data is extracted.

With this cellular assay it is possible to compare the metabolism of different substrates within the time- and concentration limits of *in vivo* therapeutic or diagnostic DNP-NMR markers. Although the assay has been developed for ^{13}C-labeled substrates, it can, with a few modifications and in a more limited form, also be used with unlabeled substrates.

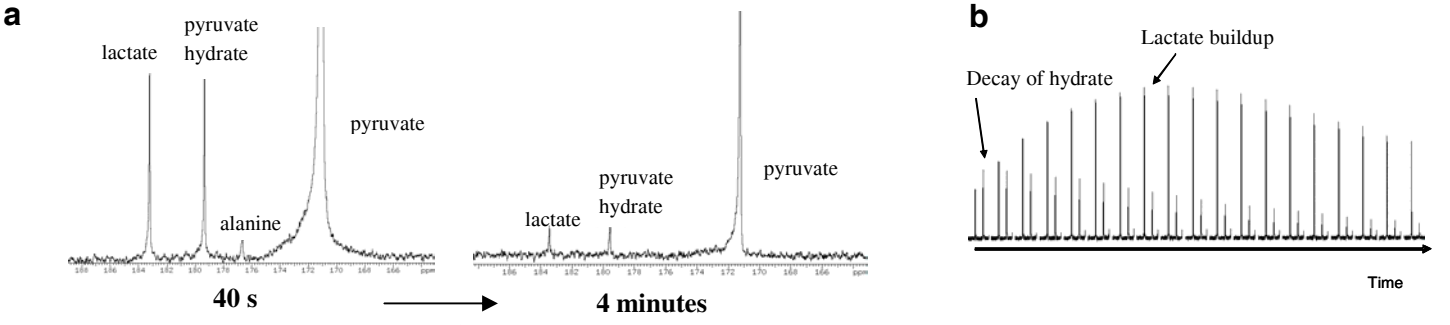

Figure 2. *^{13}C-Spectra of 1 mM pyruvate and its metabolites in liver hepatoma cells acquired after 20 seconds incubation, collected 40s after dissolution of the DNP sample (a, left), collected approx. 4 min after the DNP dissolution (a, right) and an example of experiment following the product build-up over time (b). Note that all results are obtained using one single dissolution of ^{13}C-pyruvate.*

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