

HP-¹³C-NMR detects the alternation of LDH kinetics and redox state induced by metabolite modulation

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Target audience: Researchers and physicians particularly in the fields of metabolism, nutrition, lung, and/or hyperpolarized NMR.

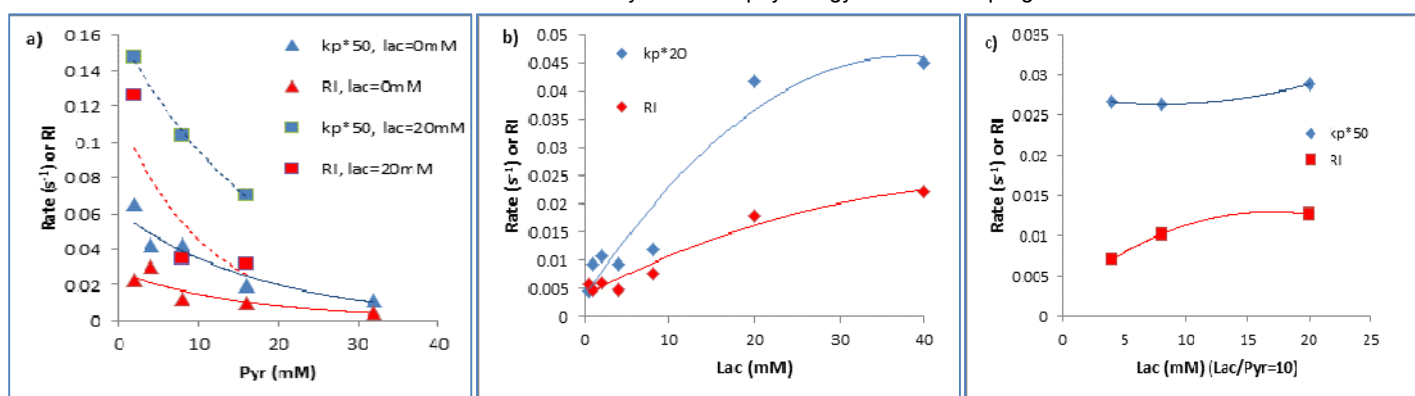
Purpose: Abnormal enzyme activities and cellular redox state have been observed in various medical conditions including diabetes, cancer, neurodegenerative diseases and lung disorders. Hyperpolarized ¹³C NMR (HP-¹³C-NMR) has been used to detect enzymatic kinetics in cells, organs, animals and human subjects. Here we aim to employ HP-¹³C-NMR to investigate non-invasively how the enzyme kinetics (rate constants) and cellular redox state may be modulated by the levels of metabolites.

Methods: The perfused rat lung system was set up in a 9.4-T Varian vertical bore NMR spectrometer, with the temperature and pH maintained at 37 °C and 7.3 ± 0.1, respectively and 400 ml PBS buffer suppl. with 3% albumin and 10mM glucose. NMR data were acquired using a 20 mm coil tuned to ¹H/¹³C/³¹P. All lungs from rats (250-300 g) were first confirmed to be viable using the criterion NTP/Pi > 1 as observed by ³¹P-NMR spectroscopy. The concentrations of pyruvate and lactate varied from 0-40 mM in the perfusate before the injection of 10-20 ml, 2-32 mM hyperpolarized 1-¹³C-pyruvate (pH=7.0, DNP method by Oxford Instruments HyperSense). The injection of the hyperpolarized tracers was completed by a constant infusion for 1-2 min. Single-pulse, non-localized ¹³C-NMR spectra were collected every 1s over a period of 300s (nominal flip angle 15°). The time course data of pyruvate and lactate were first extracted from the FID signals using a customized MATLAB® program. Ratiometric analysis¹ of the time courses of the lactate/pyruvate ratio during the phase of constant HP tracer infusion was performed on the basis of two-site exchange model, using the SNR threshold 3 for both lactate and pyruvate signals. We determined the forward (k_p) and reverse (k_i) rate constants of the lactate dehydrogenase (LDH)-catalyzed reaction and the rate constant ratio k_p/k_i . Because the LDH reaction is coupled with the redox potential NAD⁺/NADH, the ratio k_p/k_i indicates cellular redox status^{2,3} when the pH is maintained stable.

Results and Discussion: Figure a, b, and c summarize the rate k_p and redox index ($RI = k_p/k_i$) measured under various concentrations of metabolites. All data are averaged results from 2-4 lungs except for the 20mM lactate measurements in Fig. a, which were from single lungs. Also note that the rate constant k_p is displayed with a scaling factor. Fig. a shows the change in k_p and RI as the concentration of hyperpolarized ¹³C-pyruvate is modulated. The k_p decreases rapidly with the increasing concentration of hyperpolarized pyruvate when the perfusate lactate concentration is maintained at either 0 mM or 20 mM. This can be understood by the consumption and decreased levels of NADH and thus decreased forward rate constant. The cellular redox index RI shows a similar decreasing trend with increasing pyruvate concentration. Fig. b shows that k_p increases with the lactate concentration in the perfusate when the concentration of hyperpolarized pyruvate is maintained at 32 mM, and appears to reach saturation at ~25mM. This result, and the similar tendency toward increasing RI shown in Fig. b, is consistent with increased concentrations of NADH and labeling flux when the lactate pool becomes larger.⁴ We did not detect a significant trend for the reverse rate constant k_i . The rate k_i , however, is proportional to NAD⁺ concentration, which is hundreds of times that of NADH [2]; thus, a large relative change in NADH concentration does not imply a similarly large change to NAD⁺ levels. The primary effect of metabolite modulation on LDH activity is due to fast changes of the NADH levels and a new (near)-equilibrium is reached for LDH within a second.^{1,4}

We further investigated the effect of metabolite modulation on HP-¹³C-pyruvate NMR studies by perfusing lactate and pyruvate (both non-hyperpolarized) in the lung with a fixed concentration ratio lac/pyr=10. The k_p and the cellular redox index RI show little change with metabolite concentration (Fig. c). These results indicated that the redox state and enzyme kinetic rate constant have been clamped by the metabolite ratio in the perfusate. The rate k_p in Fig. c is less than those in Fig. a & b at similar lactate concentrations. This is expected because the lungs in Fig. a & b have higher lac/pyr ratios and redox indices. The forward rate constant k_p in this study is about 1-2 orders of magnitude smaller than the rate constants reported for liver or heart in the literature. This is due to the existence of a large amount of hyperpolarized ¹³C-pyruvate in the perfusate. The rates can be corrected with a volume ratio factor which is relatively constant for this well-controlled perfused system with similar lung sizes, although this correction should not change the general pattern of changes for k_p and the cellular redox index RI .

Conclusions: We have demonstrated the modulation of LDH kinetics and cellular redox state by changing the concentration of metabolites in the perfused healthy lung system. These results are consistent with the tight coupling between LDH kinetics and the cellular redox state NAD⁺/NADH. Similar approaches can be translated to other organs such as liver and heart and to in vivo studies. It may be feasible to employ LDH kinetics as an indicator of cellular redox state for the study of normal physiology and disease progression.



References:

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