Ratiometric analysis of hyperpolarized ^{13}C-NMR data to quantify reaction rate constants

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Introduction

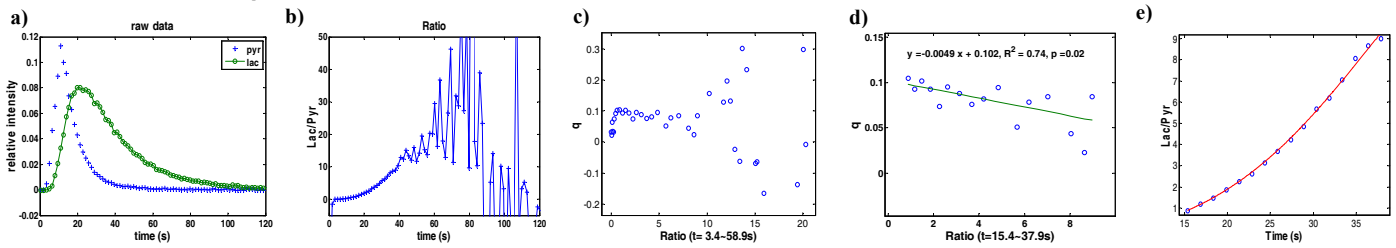
Both the kinetic model and the two-site chemical exchange model have been used to model the time courses of hyperpolarized ^{13}C-NMR signals of metabolites participating an enzymatic reaction, e.g. the LDH reaction in tissue.[1,2] These modeling methods assume that the reaction rate constants remain stable during the period of modeling. While the kinetic model parameterizes the rate of tracer injection, a mean arrival time for tracers to travel from blood to tissue, and the rate of forward reaction converting pyruvate to lactate, it neglects the reverse reaction rate from lactate to pyruvate. The two-site exchange model includes both forward and backward reaction rate constants. Because it focuses more on the period after tracers reaching the tissue, the influence of blood transport is neglected. However, the variable blood transport properties may generate the difficulty in objectively deciding the proper time range for modeling. Here we present a ratiometric analysis method to determine a suitable time range for the two-site exchange modeling. We can also quantify both forward and backward rate constants from the lactate-to-pyruvate ratio with fewer assumptions and fitting variables than previously published studies.

Theory, Materials and Methods

As described in ref. 2, let $L(t)$ and $P(t)$ stand for the NMR signal intensities of lactate and pyruvate respectively, k_l is the rate constant for conversion of lactate to pyruvate, k_p for pyruvate to lactate, ρ_l and ρ_p are the 1-^{13}C T_1 relaxation rates for lactate and pyruvate respectively. We additionally assume that $\rho_l=\rho_p=\rho$ (rigorously true in the limit of fast intracellular pool exchange). From the differential equations of the two-site exchange model, $dP/dt=-\rho P-k_pP+k_lL$ (1a); and $dL/dt=-\rho L+k_pP-k_lL$ (1b), we can derive the following relationship $q=\left(dR_{lp}/dt\right)/\left(1+R_{lp}\right)=-k_lR_{lp}+k_p$ (2), where the ratio $R_{lp}(t)=L(t)/P(t)$. If k_p and k_l are stable during the time course, $q(t)$ versus $R_{lp}(t)$ should be linear and k_p and k_l can be extracted from the slope and intercept. From the general solutions of Eq. 1 using MAPLE-14, we can also derive $R_{lp}(t)=\dfrac{r(1+R_{lp}(t_0))+\left[R_{lp}(t_0)-r\right]e^{-s(t-t_0)}}{1+R_{lp}(t_0)+\left[r-R_{lp}(t_0)\right]e^{-s(t-t_0)}}$ (3), where $r=k_p/k_l$ and $s=k_p+k_l$. $R_{lp}(t)$ can be modeled according to Eq. 3 with only two variables r and s to obtain k_p and k_l. DNP-hyperpolarized 1-^{13}C-pyruvate (~75mM, 10 µl/g body weight) was injected by tail vein into transgenic breast cancer mouse models (~10mm in diameter) in which activated HER2/Neu proto-oncogene were expressed specifically in the mammary epithelium.[3] Single-pulse ^{13}C-NMR spectra were obtained every 1.5 seconds for about 2 minutes using a home-built 11mm ^{1}H/^{13}C slot resonator on a vertical bore 9.4-T Varian NMR spectrometer. Data were post-processed by customized Matlab software. Individual peak areas of metabolites were obtained by Lorentzian fitting for each spectrum. Eq. 2 & 3 were fitted using the fitting modules in Matlab. The correlation coefficients R^2 were obtained.

Results

In figures below, typical results are shown for tumor #1. Figure a and b show the time courses of $L(t)$, $P(t)$, and $R_{lp}(t)$. Figure c depicts $q(t)$ versus $R_{lp}(t)$ and indicates that it is initially nonlinear but becomes and remains linear after t=15.4s. After t=38s, $R_{lp}(t)$ and $q(t)$ become unreliable due to low signal-to-noise. This analysis indicates stable rate constants and supports that the two-site exchange model is valid between the time limits. Thus, the ratio data in this range were fit to Eq. 2 & 3 to obtain rate constants (see Figure d & e and Table 1). The results are consistent whether modeling with Eq. 2 or 3 but the latter has more precision. The linear correlation between $q(t)$ and $R_{lp}(t)$ also supports the assumption $\rho_l=\rho_p=\rho$ for this specific time range, indicating at least the possible difference between ρ_l and ρ_p is not a significant factor here.



Tumor	time range	Fit of Eq. 2 using the 'robust fit' of Matlab				Fit of Eq. 3 using the cftool module of Matlab		
		R^2	p	k_p	k_l	R^2	k_p	k_l
#1	15.4~37.9s	0.74	0.02	0.102±0.009	0.0049±0.0018	0.998	0.099±0.003	0.0054±0.0006
#2	13.5~46.5s	0.73	0.03	0.083±0.012	0.0056±0.0024	0.996	0.080±0.002	0.0062±0.0004

Table 1

Discussion and Conclusion

In this work, we have developed for the first time a ratiometric analysis method to obtain reaction rate constants *in vivo* from hyperpolarized ^{13}C-NMR data. We identified a proper time range in which reaction rate constants are presumably stable, and modeled the lactate/pyruvate ratio data to quantify both forward and backward reaction rate constants for the LDH reaction. This method can be applied to other enzymatic reactions investigated by hyperpolarized NMR methods. The ratiometric method has the advantage of canceling out or reducing influence of those factors that are dependent on NMR spectrometers, blood transport properties, initial degree of polarization, tracer dosages, and factors which could alter metabolite T_1s *in vivo*. Derivation of reaction rate constants, which is intrinsic character of metabolic activity, is more favorable over the relative signal intensity of metabolite, SNR, and relative metabolite ratios, which are presumably instrument and/or protocol dependent.

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