Novel 31P Saturation Transfer Strategy for Measurement of Chemical Exchange Reaction Kinetics in vivo

Q. Xiong[1,2], Q. Li[3], A. Mansoor[3], F. Du[2], W. Chen[2,4], and J. Zhang[1,3]

[1]Dept. of Biomedical Engineering, University of Minnesota, Minneapolis, MN, United States, [2]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States, [3]Dept. of Medicine, University of Minnesota, Minneapolis, MN, United States, [4]Dept. of Radiology, University of Minnesota, Minneapolis, MN, United States

Introduction Magnetization saturation transfer (ST) is the most commonly used method in ^{31}P MRS to non-invasively measure the kinetics of phosphoryl-exchange reactions and thus especially suitable for *in vivo* enzyme kinetic studies such as creatine kinase (CK) and ATPase. However, the conventional ST approach is greatly limited by the lengthy data acquisition time for achieving accurate quantification of rate constant and flux. Aside from the multi-point progressive saturation experiment that retrieves apparent T_1 by curve fitting, the steady-state saturation transfer experiment alone is time-consuming enough because it usually requires a very long pre-saturation delay followed by a long saturation time in order to achieve the true steady-state magnetization. This would greatly compromise its *in vivo* applications in which scan number is large (i.e. studies requiring 3D spatial localization) or minimal scan time is preferred (i.e. patient study). Here, we propose a novel strategy called "d1opt" for improving the steady-state saturation transfer experiment, which, by employing a numerically optimized pre-saturation delay (d1opt), results in substantial reductions in saturation time as well as total repetition time.

Theory The d1opt strategy is elucidated with an example of the forward rate constant (k_f) measurement in CK reactions: PCr↔ATPγ. The ST pulse sequence usually employed consists of three segments: a pre-saturation delay (d1), frequency-selective saturation on ATPγ (d2) and 90° readout pulse followed by FID acquisition. The total repetition time (TR) approximately equals d1+d2 if acquisition time is short and negligible. The change of PCr and ATPγ magnetizations during this pulse sequence can be described by the well-known modified Block equations shown to the right, where Equations (1) and (2) apply to the d1 duration whereas Equations (3) and (4) apply during d2. The steady-state solution to Equation (3) leads to a PCr magnetization ($M_{ss,PCr}$) that is independent of its initial values ($M_{PCr}(d1)$) which in turn is determined by d1. Therefore, choosing an appropriate pre-saturation delay (d1opt) would allows us to achieve an $M_{PCr}(d1)$ very close to $M_{ss,PCr}$ in a short time and thus results in shortest saturation time (d2opt) as well as minimal repetition time. The calculation of d1opt and d2opt is based on numerical simulation of Equations (1)~(4) and depends upon an arbitrarily estimated k_f range and accuracy level using known intrinsic T_1 values.

$$\frac{dM_{PCr}(t)}{dt}=\frac{M_{0,PCr}-M_{PCr}(t)}{T_{1,PCr}}-k_f M_{PCr}(t)+k_r M_{ATP\gamma}(t) \quad (1)$$

$$\frac{dM_{ATP\gamma}(t)}{dt}=\frac{M_{0,ATP\gamma}-M_{ATP\gamma}(t)}{T_{1,ATP\gamma}}+k_f M_{PCr}(t)-k_r M_{ATP\gamma}(t) \quad (2)$$

$$\frac{dM_{PCr}(t)}{dt}=\frac{M_{0,PCr}-M_{PCr}(t)}{T_{1,PCr}}-k_f M_{PCr}(t) \quad (3) \qquad M_{ATP\gamma}(t)=0 \quad (4)$$

Experiment The experiments were performed at a 40-cm bore 4.7 T magnet with 4 healthy farm pigs. *In vivo* ^{31}P MR spectra from the anterior left ventricle (LV) wall were obtained with an open-chest surgery and a surface coil sutured onto it as described previously [1]. A BISTRO pulse train was used to saturate the ATPγ followed by an adiabatic half passage pulse (AHP) for readout [2,3].

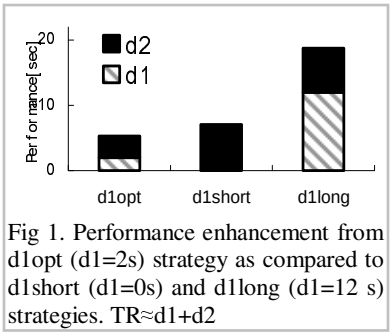

Fig 1. Performance enhancement from d1opt (d1=2s) strategy as compared to d1short (d1=0s) and d1long (d1=12 s) strategies. TR≈d1+d2

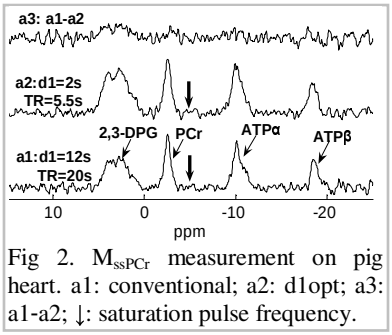

Fig 2. M_{ssPCr} measurement on pig heart. a1: conventional; a2: d1opt; a3: a1-a2; ↓: saturation pulse frequency.

Results The d1opt and d2opt for an estimated k_f range of 0.2~0.8 s^{-1} (valid for most *in vivo* and *in situ* myocardial CK kinetics studies based on previous reports) and accuracy level of >95% were calculated to be 2 and 3.3 s, respectively. This is significantly superior to non-optimized approaches (both long and short d1, shown in Fig 1) in regards to saturation time and total repetition time. The experimental validation of d1opt strategy on pig heart is shown in Fig 2. There's no detectable difference in M_{ssPCr} measurement between the d1opt and the conventional strategies whereas great reductions in saturation time (56%) and total repetition time (73%) have been granted for d1opt approach. A further quantitative validation was performed using progressive saturation experiments with different d1 values. The normalized PCr signal intensities together with simulated lines are shown in Fig 3. The excellent consistence between experiment and simulation results again confirms the validity of d1opt strategy. The k_f value calculated by using the new strategy yields a value of 0.42±0.01 s^{-1} which is similar to our previous reports of 0.49±0.07 s^{-1} in pig hearts using the conventional method [1].

Discussions The estimated k_f range is based on reports of previous *in vivo* or *in situ* studies. In general, the narrower the estimated k_f range is, the shorter a saturation time as well as repetition time can be granted, whereas accompanied with greater risks and *vise versa*. Considering usually a very small standard deviation in k_f measurement, a much narrower k_f range can be confidently estimated after initial few experiments and thus further shorten the d2 and TR. In addition, detailed simulation reveals that the d1opt strategy is quite robust for variations of parameters used for calculation such as PCr/ATPγ and intrinsic T_1. Another unique advantage of d1opt strategy over other strategies is the minimal saturation time. This will significantly reduce safety concerns for human studies and also benefit in reducing RF spillover effects. Using the BISTRO saturation pulse, the spillover effect was undetectable with d1opt strategy in our experiment such that control spectra usually required in conventional approach can be eliminated. Finally, d1opt strategy is readily applied to more complicated reaction models such as three-site exchange system (PCr↔ATPγ↔Pi) usually utilized in bioenergetic studies of rodent heart or brain.

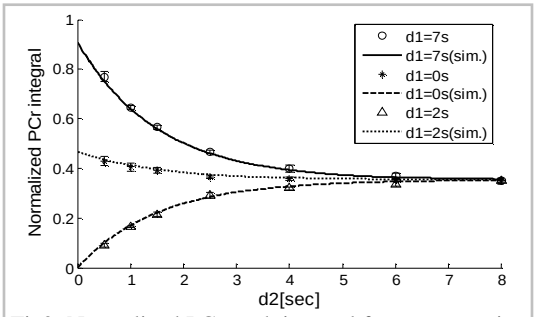

Fig3. Normalized PCr peak integral from progressive saturation experiments with different d1 values on 4 pig hearts. Data were fit into equation (3) to calculate k_f and T_1 that were further used to plot the simulated lines.

Reference: [1] Ye Y, *et al.*, Circulation, 2001. [2] de Graaf RA, *et al.*, J. Magn. Reson., 1996. [3] Lei H, *et al.*, PNAS, 2003.

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