

Quantifying Exchange rates in CEST Agents using saturation time and saturation power dependencies of the magnetization transfer effect (QUEST and QUESP)

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INTRODUCTION

Chemical Exchange Saturation Transfer (CEST) is a new contrast mechanism based on proton exchange between contrast agents and water^{1,2}. The signal intensity changes in CEST experiments depend on a multitude of parameters, including agent concentration, number of exchangeable protons, proton exchange rate, T1, saturation time, and saturation power. Of these, the chemical exchange rate is often the parameter of interest that reflects tissue pH and the molecular environment. Existing spectroscopy methods to measure exchange rates of the agent, such as the Water EXchange (WEX) sequence³, are time consuming for the lower rates due to the low contrast agent concentration, and not suitable for measuring the faster rates that occur for CEST agents at high pH. In this work we present two techniques to measure fast chemical exchange rates via water detection. Both methods, Quantifying Exchange using Saturation Time (QUEST) and Quantifying Exchange using Saturation Power (QUESP), were applied on one representative CEST agent, Poly-L-Lysine (PLL, 705.8 kD).

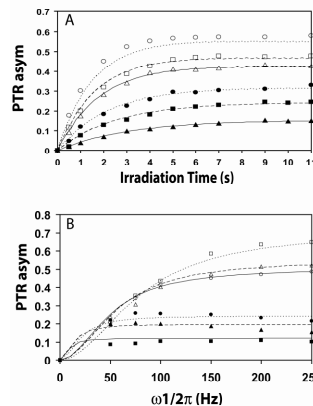
METHODS

All measurements were performed on an 11.7T Bruker Avance system with triple axis gradients at 310K. The WEX pulse sequence was used as described previously³. QUEST and QUESP experiments consisted of a frequency-selective saturation pulse with variable frequency, power and time followed by spin echo MRS (TR/TE = 16s/2ms) for water detection. Curves were fit using Matlab's nonlinear fitting routines. For QUESP and WEX experiments, analytical solutions were used to extract the exchange rates. For QUEST experiments, numerical solutions to the Bloch equations were carried out using the Ordinary Differential Equation solver. The results were fed into a nonlinear fitting routine with the experimental data. We assumed no change in relaxation times of the amide or water protons for all pH values. Error analysis was carried out using the F statistic. The pH dependence of amide proton exchange rate was investigated by: 1) Monitoring the linewidth as a function of pH, 2) WEX experiment, 3) QUESP and 4) QUEST. For the linewidth measurement, spectra were collected using a $\pi/2$ -detect pulse sequence. We extracted the chemical exchange rate from these linewidths by using the formula $LW=(k_{sw})/\pi$. For QUEST and QUESP the change in NMR signal was calculated using the expression for the asymmetric Proton Transfer Ratio (PTRasym) which is $PTRasym = \frac{I^+ - I^-}{I^0}$ where I^+ , I^- are the MR signal intensities post saturation at +3.6 ppm (amides), -3.6 ppm (reference) from the water frequency and I^0 without saturation. This evaluates the intensity loss as a function of saturation frequency.

RESULTS AND DISCUSSION

QUEST was used to measure the exchange rate of the CEST agent Poly-L-Lysine (PLL). For low exchange rates (at lower pH) the experimental data was collected using a saturation pulse of 100Hz, which has a direct effect on the water proton (spillover) that is smaller than 2%. Therefore the spillover can be neglected and the data can be fit using 4 Bloch equations. For exchange rates higher than 400 Hz (at high pH) a stronger saturation pulse should be used to ensure proper labeling, in which case the spillover effect is not negligible anymore and the data should be fit using 6 Bloch equations. The fits are in good agreement with the experimental data, as can be seen below in the figure comparing the experimental QUEST data vs. best fits (A) for PLL over a range of pH's. QUEST has the advantage that a steady state doesn't have to be reached by the saturation pulse, and so data can be taken with short TR.

For QUESP experiments, the analytical solutions should be used, which rely on a steady state being reached. The data can also be reliably fit (Figure B), however TR needs to be quite long ($\sim 5T_1$) which might prove problematic for use in vivo. Both QUESP and QUEST rates are in good agreement with WEX and linewidth-based rate estimates (Table). For higher exchange rates, WEX is less accurate due to the speed of the transfer, which causes slight changes in buildup to result in large changes in best fit exchange rate. In addition, for the faster exchange rates the QUESP method is less accurate. For example, for pH 7.9, 7.7, 7.3 all of the data collected with 100Hz saturation power or



Exchange rate(Hz) for PLL at various pH's

pH	LW ^a	WEX ^b	QUEST ^b	QUESP ^b
7.9	1250	-	1289±220 ^c	1345±231
7.7	678	-	610±71 ^c	635±73
7.3	374	400±125	502±60 ^c	570±62
6.7	138	120 ±13	277±30	183±30
6.5	110	86 ±8	115±9	99±15
6.0	78	36±1	69±3	60±9

- This was derived using the formula $LW=(k_{sw})/\pi$.
- Errors were obtained using the F statistic and the 95% confidence limits
- Obtained using 200Hz saturation power and fit to 6 equations including spillover

below are equivalent, because of incomplete saturation before transfer. Therefore only a few data points determine the rate. The results in the Table show that PLL is a very sensitive pH agent in the physiological range. Using the data in the table as a calibration, it should be feasible to predict experimental parameters for imaging and spectroscopy of this CEST contrast agent in vivo.

CONCLUSIONS

We report two methods to determine exchange rates using the water signal. These rates can be used to quantify or calibrate pH dependence, as shown for PLL, which should to be a sensitive pH agent in the physiological range. It should also be possible to use these approaches to quantify magnetization transfer rates in conventional MT imaging.

(1) Ward, K. et al., *J. Magn. Reson* **2000**, *143*, 79. (2) Goffeney, N. et al., *J Am Chem Soc* **2001**, *123*, 8628. (3) Mori, S. et al. *J. Magn. Reson B* **1996**, *110*, 96.