Chemical Exchange Saturation Transfer Effects from Creatine Kinase Reaction Metabolites

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Introduction:
Phosphocreatine (PCr), adenosine triphosphate (ATP) and creatine (Cr) serve as a major energy sources in biological systems. Creatine kinase (CK), an important enzyme in the biological system, catalyses the reversible conversion of Cr into PCr by utilizing ATP. Non-invasive measurements of these metabolites *in vivo* both in normal and pathological conditions have been performed using magnetic resonance spectroscopy (MRS). ^{31}P MRS has been used to quantify PCr and ATP and to probe the kinetics of the CK reaction and ^{1}HMRS has been widely used to quantify the total Cr concentration (Cr + PCr), MRS techniques can provide accurate concentration of these metabolites *in vivo* but are limited to low resolution. There is no currently available ^{1}HMRS method, which can quantify and differentiate Cr from PCr. Here, we explore the chemical exchange saturation transfer (CEST) effect from these metabolites in phantoms based on their exchangeable amide (-NH) and amine (-NH_2) protons. In the current study, using high resolution non water suppressed ^{1}HMRS first we identified the exchangeable peaks from Cr, PCr, ATP and ADP phantoms. Second, the exchange rate and logarithmic dissociation constant (pKa) of these peaks were measured. Finally, based on the exchange rate information CEST imaging was performed on phantoms of Cr, PCr, and ATP at their physiological concentrations. We found that with proper choice of experimental parameters, one can obtain high resolution Cr CEST (CrEST) maps with negligible contribution from PCr, and ATP.

Materials methods:
100mm solutions of Cr, PCr, and ATP were prepared in PBS and 99.9% D_2O. High resolution ^{1}HMRS was performed at different temperatures (10, 25 and 37 °C) using single pulse acquisition in a 9.4T vertical bore NMR spectrometer with a 5mm NMR probe (Varian, Palo Alto, CA). The exchange rates from these metabolites were determined using the equation: $LW = 1/\pi T_2 + k_{ex}/\pi$, where LW is line width of the exchangeable peak, T_2 is transverse relaxation time and k_{ex} is the exchange rate. The measurement of T_2 was performed at 10 °C using spin echo acquisition with different echo times. The calculated T_2 value was used to measure the k_{ex} for Cr, PCr, and ATP at 37 °C. We also measured the pKa (-log Ka, where Ka is dissociation constant) values of exchangeable proton from each metabolite at 37 °C. To measure the pKa value, 100mM solution of each metabolite was prepared at different pHs (3,4,5,6,7,8,9,10,11 and12) and high resolution ^{1}HMRS was performed. The area of the exchangeable peaks were calculated and plotted as a function of pH (Fig. 2). The resulting sigmoid curves were fitted using s-curve function and the point of maximum derivative was identified as pKa value. For CEST imaging studies, 10mM solutions of Cr, PCr, and ATP were prepared in PBS at pH 7.0. These samples were added to small test tubes (10mm diameter), and immersed inside a large PBS phantom. CEST imaging was performed on 3T Siemens whole body MRI scanner (Siemens Medical Systems, Erlangen, Germany). During the course of experiment temperature was maintained at 37±1^{0}C using a custom designed stryrofoam chamber. The sequence parameters were: slice thickness =10 mm, GRE flip angle=10^o, GRE readout TR=5.6 ms, TE =2.7 ms, field of view =100×100 mm^2, matrix size =192×192, and one saturation pulse and 64 segments acquired every 10 s. For saturation, a pulse train with a saturation pulse amplitude (B_{1rms}) 155 Hz and a total duration of 1s was used with 10 Hanning windowed rectangular pulses of 99.8 ms duration each with a 0.2 ms delay between them. The CEST contrast at 1.8 ppm was calculated using the method described in detail previously after B_0 and B_1 correction (1).

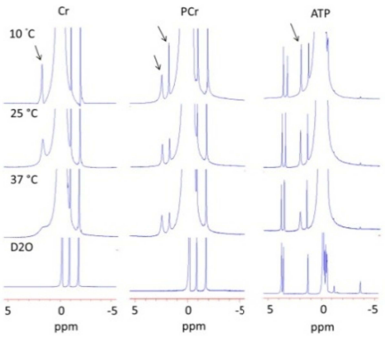

Figure 1: High resolution NMR spectra showing sharp amine proton resonances from Cr (1.8ppm), PCr (2.5ppm and ~1.8ppm) and ATP (2.0ppm) at lower temperatures which broadened at higher temperature due to exchange broadening. Disappearance of these peaks in D2O confirms these peaks are exchangeable peaks.

Results and Discussion:
Well-resolved –NH_2 proton resonances from Cr (1.8 ppm.), PCr (2.5 ppm. and ~1.8 ppm), and ATP (2.0 ppm) are visible in the ^{1}HNMR spectra at lower temperatures < 25 °C (Fig.1). These peaks broaden as the temperature is increased. At 37 °C, while Cr –NH_2 protons exhibit a broad resonance, the –NH_2 protons resonances from PCr, and ATP still show clear and sharp peaks. The disappearance of these resonances in D_2O confirmed that these peaks are from exchangeable protons. The exchange rates (k_{ex}) were determined from Cr (950 s^{-1}), PCr (140s^{-1} for 2.5ppm peak, and 120 s^{-1} for 1.8ppm peak), and ATP (115 s^{-1}). The higher exchange rate in the Cr suggests the higher dissociation constant of its labile protons. In PCr, the pKa for the exchangeable peak at ~2.5ppm was 7.96 while for the exchangeable peak at ~1.8ppm it was 8.14. The pKa values for the ATP (~2.0ppm) and Cr (~1.8ppm) exchangeable peaks were 8.17 and 6.6 respectively (Fig. 2). Low pKa (-log ka) value of Cr –NH_2 protons suggests its faster dissociation and hence its faster exchange with water spins compared to PCr and ATP. Compared to the chemical shift difference at 3T [Cr; $\Delta\omega$ = 1300 rad s^{-1}] the exchange rate of Cr amine protons is in slow to intermediate exchange regime while it is in slow exchange regime for PCr and ATP (PCr; $\Delta\omega$ = 1900 rad s^{-1} and ATP: $\Delta\omega$ =1500 rad s^{-1}). Z-spectra at 3T showed a broad resonance from Cr –NH_2 proton which centered at ~1.8ppm (Fig.3). The broadness in the Cr could be due to the higher exchange rate of Cr –NH_2 protons. Figure 4 A shows the CEST map from Cr, PCr, and ATP at 1.8ppm at 3T. Predominant CEST contrast was observed from Cr. The graph (Fig.4 B) shows a linear relationship between CrEST and Cr concentration with a slope of 0.6% per mM. The optimal B_{1rms} for CrEST mapping with negligible contribution from PCr, and ATP was 155 Hz (Fig.4 C). The slow exchange rate of amine protons in PCr, and ATP preclude their CEST detection with these saturation pulse parameters. High resolution proton imaging of Cr is possible without any contamination especially from PCr, which may provide a new imaging method to detect the changes in Cr as well as monitor the kinetics of CK reaction *in vivo* in various pathological conditions.

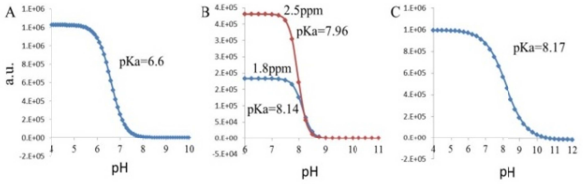

Figure 2: The fitted curves show the plotted integration value of exchangeable peak from Cr (A), PCr (B) and ATP (C) as function of pHs. The calculated pKa values for each metabolite are shown in figure intersect.

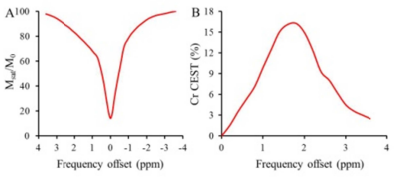

Figure 3: Z-spectra (A) and Z-spectra asymmetry curve (B) show a broad resonance from Cr –NH2 proton which center at ~1.8 ppm.

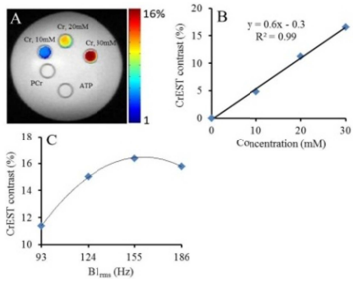

Figure 4: A: CEST map at 1.8ppm shows the predominant CEST contrast from Cr. B: shows the linear relationship between [Cr] and Cr CEST. C: graph represents the B_{1rms} dependent CrEST.

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References:
1. Haris M et al. Neuroimage 2011;54:2079-85.