A 31P Magnetization Transfer Approach for Studying High-energy Phosphate Metabolic Rates, Fluxes and Thermal Equilibrium in Three-site Chemical Exchange System of PCr,ATP and Pi in Human Brain

F. Du[1], X-H. Zhu[1], H. Qiao[1], X. Zhang[1], W. Chen[1]

[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, Minnesota, United States

Introduction One fundamental chemical process occurring in the brain is the cerebral ATP metabolism (via ATPase) which controls the biochemical energy generation through ATP synthesis and ATP utilization for supporting brain function and activation. The ATP metabolism is also tightly coupled to the PCr metabolism via the creatine kinase (CK). It is essential to establish a noninvasive approach for studying these chemical processes in the human brain *in vivo*. Although several ^{31}P MRS magnetization transfer (*MT*) methods such as conventional two-site saturation transfer (*CST*) (1), inversion transfer (*IT*) (2), Multiple-site saturation transfer (*MST*) (3) as well as 2D chemical exchange spectroscopy (*2D-EXSY*) (4) had been proposed to measure the chemical exchange rate constants and fluxes in the three-site chemical exchange system of (PCr↔ATP↔Pi), many of them pose technical limitations for precisely determining these kinetic parameters *in vivo*. For instance, the three-site system was treated as two independent two-site systems (i.e., PCr↔ATP and ATP↔Pi) in the most of literature based on the *CST* and *IT* methods; *2D-EXSY* has a low detection sensitivity; and the *MST* approach was based on the hypothesis of CK and ATP equilibriums. These limitations have resulted in some controversies. One mystery was regarding to the observation based on *CST* measurements showing an inequality of the forward and reverse fluxes of CK reaction in the brain. We have proposed a comprehensive and accurate ^{31}P MT approach, which does not require the assumption of the thermal equilibriums, for robustly determining the forward and reverse chemical exchange rates in the presence of multiple-site reactions. This approach has been applied to study the energy transfer fluxes of CK and ATPase in the human vision cortex at 7 tesla.

Theory and Methods The chemical reactions among PCr (referred as ***a***), ATP (referred as ***b***) and P_i (referred as ***c***) and their Bloch equations account for chemical exchanges are given as following:

$$\mathrm{PCr}\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}\mathrm{ATP}\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}\mathrm{P_i}\qquad \boldsymbol{a}\quad\quad \boldsymbol{b}\quad\quad \boldsymbol{c}$$

$$dM_a/dt=-(M_a-M_a^0)\cdot T_{1a}^{-1}-k_1M_a+k_{-1}M_b \quad (1a)$$

$$dM_b/dt=-(M_b-M_b^0)\cdot T_{1b}^{-1}-k_{-1}M_b-k_2M_b+k_1M_a+k_{-2}M_c \quad (1b)$$

$$dM_c/dt=-(M_c-M_c^0)\cdot T_{1c}^{-1}-k_{-2}M_c+k_2M_b \quad (1c)$$

$$k_{-1}=\alpha_a\left(\frac{M_a^*}{M_a^0}-\frac{T_{1a}^{-1}}{\alpha_a}\right)*\frac{M_a^0}{M_b^*} \quad (2a)$$

$$k_1=\alpha_b\left[\frac{M_b^*}{M_b^0}-\frac{T_{1b}^{-1}}{\alpha_b}\right]*\frac{M_b^0}{M_a^*} \quad (2b)$$

$$k_2=\alpha_c\left[\frac{M_c^*}{M_c^0}-\frac{T_{1c}^{-1}}{\alpha_c}\right]*\frac{M_c^0}{M_b^*} \quad (3a)$$

$$k_{-2}=\alpha_b\left[\frac{M_b^*}{M_b^0}-\frac{T_{1b}^{-1}}{\alpha_b}\right]*\frac{M_b^0}{M_c^*} \quad (3b)$$

When ***b*** is fully saturated, k_1, k_{-2} and apparent α as well as intrinsic spin-lattice relaxation rate (T_1^{-1}) of ***a***, ***c*** can be unambiguously determined because the three-site system (***a***↔***b***↔***c***) can be treated as two two-site systems (***a***↔***b*** and ***b***↔***c***). While ***c*** is saturated and ***a***, ***b*** approach to steady states, Eqs. (2a) and (2b) can be derived. In the similar way, k_2 and k_{-2} can be deduced from Eqs. (3a) and (3b) by saturating ***a***. α and T_1^{-1} of ***b*** can be calculated from Eqs. (2b) and (3b). Therefore, four ^{31}P MR spectra were acquired for determining all parameters involving Eqs. (2) to (4) if we assume T_1 of a, c are constant and available. This proposed approach was applied and tested in the human visual cortex using a 5-cm-diameter surface coil at 7 tesla and fully relaxed condition. B_1-insensitive selective train (BISTRO) was used to saturate γ-ATP, PCr and Pi, respectively. The results present mean and standard deviation (n=7-12).

$$\alpha_a=(k_1+T_{1a}^{-1}) \quad (4a)$$

$$\alpha_b=(k_2+k_{-1}+T_{1b}^{-1}) \quad (4b)$$

$$\alpha_c=(k_{-2}+T_{1c}^{-1}) \quad (4c)$$

Results and Discussion Figure 1 illustrates the four ^{31}P spectra acquired from one representative subject based on the proposed MT approach. The saturation of any one of three resonance peaks (i.e., PCr, Pi and ATP) can lead to the signal reduction in other two resonance peaks (e.g., see Figs. 1c and 1d). This result clearly reveals a three-site chemical exchange system *in vivo*. The difference spectrum in Fig. 1e demonstrates that only the intracellular Pi (4.83 ppm, pH=7.04) exchanges with γ-ATP with an exchange rate that can be detected by ^{31}P MT measurement. The small shoulder peak at 5.26 ppm, which is usually assigned to extracellular Pi or other phosphate metabolite, doesn't change when saturating γ-ATP or PCr. Thus, it was excluded for the quantification of ATP fluxes.

The forward chemical exchange rate constants to ATP from PCr and P_i were 0.33±0.04 s^{-1} and 0.18±0.05 s^{-1}, respectively, as well as T_1 of a, c were 4.86s and 3.77s based on the MT experiment in the presence of γ-ATP saturation. The reverse chemical exchange rate constants from ATP to PCr and P_i were 0.62±0.06 s^{-1} and 0.11±0.03 s^{-1} if the spin system was treated as two-site exchange systems (i.e., *CST* approach). The forward and reverse flux ratios of CK and ATPase reactions were determined as 0.71±0.13 and 0.49±0.15 based on these rate constants, leading to the non-equilibrium conclusions. In contrast, by using the proposed approach for treating three-site system, the reverse chemical exchange rate constants from ATP to PCr and P_i were determined as 0.47±0.05 s^{-1} and 0.05±0.01 s^{-1}, ultimately, the flux ratios of CK and ATPase reactions were 0.93±0.12 and 1.08±0.21, indicating that both CK and ATP reactions are in equilibrium in the human visual cortex. Additionally, the apparent spin-lattice relaxation time of ATP was determined as 0.68±0.07 s by using Eqs. (2b) and (3b), which was in excellent agreement with the result (0.66±0.12 s) obtained independently by the inversion recovery experiments with PCr and P_i simultaneously saturated. This further supports and validates our proposed approach.

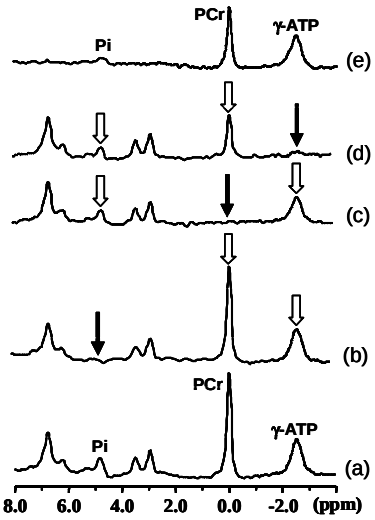

Fig.1. ^{31}P spectra acquired from a normal subject in the absence (a) and presence of fully saturation on the resonance of P_i (b), PCr (c) and γ-ATP (d), respectively. (e) Difference spectrum of (a)-(d). Saturation time was 7.6s. The narrow arrows present the saturation sites and the wide arrows indicate the signal reductions due to the magnetization transfer.

In summary, the coupled chemical reactions involving the PCr↔ATP↔Pi system has to be treated as a three-site chemical exchange system, and all four chemical reaction rate constants and their fluxes can be unambiguously determined by the four proposed ^{31}P spectra. The measurement can be further simplified to two ^{31}P spectra measurement if the equilibrium conditions are applied. This approach should be very useful to study cerebral bioenergetics *in vivo* at both physiological and pathological conditions (5).

References (1) Forsen, *JCP*,1963;39:2892. (2) Degani et al., *BioChem*,1985;24:5510. (3) Ugurbil, *JMR*,1985;64:207. (4) Balaban et al., *JBC*, 1983;258:12787. (5) Lei et al., *PNAS*, 2003;1000:14409.

Acknowledgments NIH grants of EB00329, NS41262, NS38070, NS39043, EB00513 and P41 RR08079, the W.M. Keck Foundation and the MIND institute.