^{31}P Magnetization Inversion Transfer Study of Three-site PCr←→ATP←→Pi Exchange System in Human Brain at 7T

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Introduction Magnetization inversion transfer (IT) [1] as a non-invasive NMR approach has been used for studying the creatine kinase (CK) reaction, *i.e.* PCr↔ATP, in the human and animal. To date, the IT measurement was based on the two-site chemical exchange model in which ATPase reaction (ATP↔Pi) was ignored. This simple model has raised some concerns regarding the accuracy for measuring the forward and reserve fluxes involving in this chemical exchange system because it led to some conflicted results: equality [1] or inequality [2] of forward and reserve fluxes for CK reaction. However, in principle, the chemical reactions of CK and ATPase constitute a three-site chemically coupled spin system, *i.e.* PCr↔ATP↔Pi. In this study, we used three-site chemical exchange model and full matrix Bloch equation analysis method to investigate the complication of the three-site versus two-site chemical exchange models on the determination of the metabolic fluxes for the ATPase and CK reactions.

Method For the three-site chemical exchange system, the dependence of magnetizations on the inversion recovery time can be described by Equation (1). In this equation, A is a kinetic matrix and $\vec{M}$ is the magnetization vector, in which three components equal to the longitudinal magnetizations of PCr (a), γ-ATP (b) and Pi (c), respectively. $\vec{M}^0 = \vec{M}(t=\infty)$ presents the matrix of the longitudinal magnetizations of PCr, γ-ATP, Pi under complete recovery condition. Equation (2) is the general solution of Equation (1), where I is a 3×3 unity matrix and $\vec{M}(0)$ is the initial magnetization condition for Equation (1) at t=0. Equation (2) can be simulated numerically. The results were compared to the outcomes derived from the established two-site exchange model according to the following Equations (3a) and (3b) [1]:

$$\mathrm{PCr} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ATP} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{Pi} \qquad \begin{matrix} \mathrm{a} & & \mathrm{b} & & \mathrm{c} \end{matrix}$$

$$\frac{\partial \vec{M}(t)}{\partial t} = \mathrm{A}\left[\vec{M}(t) - \vec{M}^0\right] \quad (1)$$

$$\mathrm{A} = \begin{Bmatrix} -(T_{1a}^{-1}+k_1) & k_{-1} & 0 \\ k_1 & -(T_{1b}^{-1}+k_{-1}+k_2) & k_{-2} \\ 0 & k_2 & -(T_{1c}^{-1}+k_{-2}) \end{Bmatrix}$$

$$\vec{M}(t) = (I - e^{t\mathrm{A}})\vec{M}^0 + e^{t\mathrm{A}}\vec{M}(0) \quad (2)$$

$$M^i(t) = M^i(\infty) + c_1 \exp(\lambda_+ t) + c_2 \exp(\lambda_- t) \qquad (3a)$$

$$M^u(t) = M^u(\infty) + c_3 \exp(\lambda_+ t) + c_4 \exp(\lambda_- t) \qquad (3b)$$

where, M^i and M^u are the inverted and undisturbed magnetizations, respectively. c_1, c_2, c_3 and c_4 as well as $\lambda_\pm$ are functions of the chemical exchange rates and longitudinal relaxation times of the inverted and non-inverted nuclei [1].

Experiments The experiments were performed at a 90-cm bore 7T magnet. *In vivo* ^{31}P spectra from human occipital lobe were acquired under full relaxation condition. Hyperbolic Sech inversion RF pulse (180 Hz exciting band) was used to selectively invert PCr or γ-ATP resonance peak.

Results and Discussions Figure 1 demonstrates the *in vivo* ^{31}P spectra acquired from the human occipital lobe when the γ-ATP resonance peak was selectively inverted with a varied inversion recovery time. We had measured all the chemical exchange rate constants and intrinsic spin-lattice relaxation times of the chemical exchange of PCr↔ATP↔Pi by the newly developed MSS approach in the human brain [3]. Therefore the magnetization recovery time courses were simulated by Equation (2) and (3), respectively, using these known kinetic parameters, when chemical reactions were treated as a three-site or two-site chemical exchange system. Here we only presented results with γ-ATP inversion (see Fig. 2), which has the similar results with PCr inversion. Figure 2A indicates that in the brain of healthy subject the magnetization differences for the PCr and γ-ATP recovery time courses could be ignored when the CK reaction was treated as either a three-site or two-site chemical exchange system, so that it can be approximately treated as a two-site chemical exchange system practically. This is attributed by the fact that the ATPase fluxes were five times smaller than the CK fluxes, thus, less influence on the determination of CK reaction fluxes [3]. With the increased chemical exchange flux of ATPase reaction which could result from the elevated chemical exchange rate or intracellular Pi concentration, ATPase reaction may need to be taken into account for accurately measuring the CK fluxes (data not shown herein). In contrast, ATPase reaction must be treated as a three-site chemical reaction system in order to accurately measure its chemical reaction fluxes as illustrated by Fig. 2B. Therefore, our simulations clarify two debated questions: whether CK reaction can be treated as a two-site chemical exchange reaction for IT experiments (our answer: yes); and whether inequality of forward and reverse fluxes of CK reaction deduced by the IT approach in the literatures is caused by the approximation of two-site chemical exchange model (our answer: no).

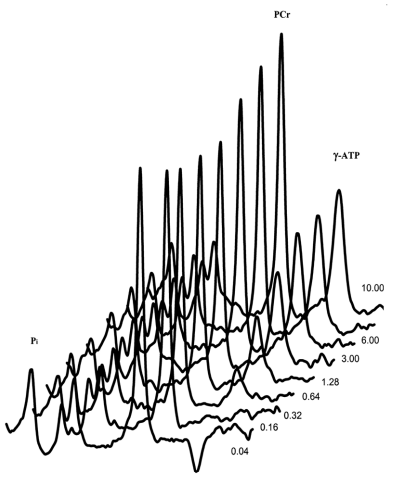

Fig.1 *In vivo* ^{31}P spectra acquired with the varied inversion recovery time after γ-ATP was selectively inverted.

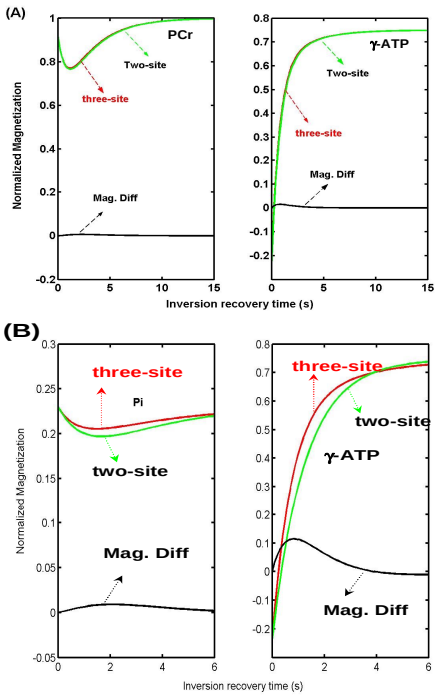

Fig.2 Magnetization recovery time courses and differences predicted by the three-site and two-site chemical exchange models for the γ-ATP inversion recovery experiments for (A) CK reaction and (B) ATPase reaction. The red and green lines were predicted by the three- and two-site chemical exchange model, respectively. The black lines are the magnetization differences predicted by the three- and two-site chemical exchange model

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Reference: 1. Degani H. et al. *Biochemistry*, 1985; 2.Joubert F. et al. *Mol Cell Biochem* 2004; 3. Du F. et al. *MRM* 2007.