The exchange pathways of NOE-CEST as revealed by NMR study

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Target audience: Investigators interested in Amide Proton Transfer (APT) and relayed Nuclear Overhauser Enhancement (rNOE) CEST imaging.

Purpose: Chemical exchange saturation transfer (CEST) can be used to enhance the signal of exchangeable protons of low-concentration solutes through indirect detection via the water protons. Endogenous mobile proteins contain a high concentration of exchangeable amide protons, which can be used to detect pH changes or tumors *in vivo*. Such amide Proton Transfer (APT) imaging is usually performed by acquiring a difference image of saturation at 3.6 ppm (amide proton saturation) and -3.6 ppm (reference image) to remove the direct water saturation and the conventional magnetization transfer contrast (MTC). Unfortunately several types of aliphatic protons in proteins resonate at around -3.6 ppm, and their NOE signals complicate the interpretation of APT images [1]. On the other hand, the aliphatic proton NOE itself has potential for *in vivo* applications due to its high signal intensity [2,3]. However, the exact mechanism of the magnetization transfer among water, amide protons and aliphatic protons in mobile proteins is still under debate [4] with some investigators indicating direct through-space dipolar coupling for the NOEs [1,4], and others suggesting relayed NOEs [2,5]. In this work, a series of NMR experiments were designed to reveal the magnetization transfer pathways among these three pools using an egg white phantom. The current findings will be important to understand APT and NOE-CEST images and, potentially, to develop new contrast.

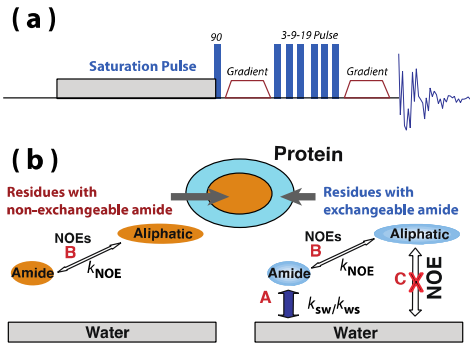

Fig 1: (a) WATERGATE sequence with water saturation pre-pulse. (b) magnetization transfer pathways and the exchange rates of water, amide and aliphatic protons.

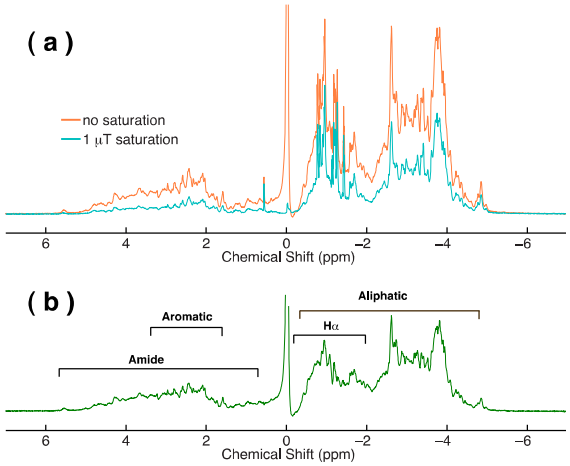

Fig 2: (a) typical spectrum of egg white acquired with and without water saturation pulse. (b) spectrum of exchangeable protons by subtracting the above two spectra.

Method: MRI experiments were performed on a vertical bore 750MHz Bruker NMR system equipped with a double channel broadband NMR probe. High-resolution proton NMR spectra of an egg white phantom were recorded using a 3-9-19 WATERGATE sequence segment (3.4 ms echo time) for water suppression (Fig. 1a). Signal that arises only from the water exchangeable protons was measured as the difference of spectra recorded with and without a 2 s water saturation pulse (1 μT) before the WATERGATE segment. The magnetization transfer pathway was investigated by monitoring the dependence of the signal reduction of the amide protons (3.6 ppm) and aliphatic protons (-3.6 ppm) on the water saturation pulse length.

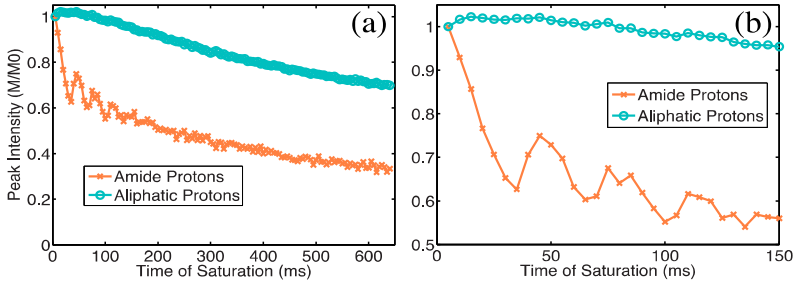

Fig 3: (a) time dependence of amide protons and aliphatic protons on water saturation duration; (b) expansion of (a) in the time range of 0 to 150 ms.

Results and Discussion: The typical proton spectrum in egg white covers the region from -5 to 5 ppm with respect to water resonance (Fig. 2a). Some sharp peaks, most likely from small metabolites, can be seen in the region of 0 to -2 ppm (Fig. 2b). It indicates that these peaks from small molecules are not exchanging with water. Fast exchanging protons such as amine and OH groups together with the small MTC pool are not detectable by WATERGATE, but the amides are visible downfield (higher frequency from water).

The dependence of the signal intensities of amide protons and aliphatic protons on the water saturation pulse length is plotted in Fig. 3(a). In the time range of 50 ms (Fig. 3b), saturation of water almost has no effect on aliphatic proton signal intensities, while a loss of about 40% of amide proton signal intensity occurs. This is a clear evidence that any direct coupling between aliphatic protons and water protons is negligible for this phantom (Pathway C in Fig. 1b). When the saturation time is longer than 50 ms, the aliphatic proton signal begins to decrease due to relayed transfer through the amide proton pool, which shows a pattern similar to relayed NOE (rNOE), well studied in high resolution NMR [5,6].

Conclusion: Water saturation experiments show negligible direct transfer to aliphatic protein protons in egg white phantoms, a well-studied model for biological tissue. This work confirms that the magnetization transfer from water to aliphatic protons is relayed through amide protons.

References:[1]. Ling W et al. PNAS 2008 105:2266. [2] Jones CK et al. MRM 2012 67:1579 and Neuroimage 2013 15:114. [3]. Xu J et al. MRM DOI: 10.1002/mrm.24850. [4] Jin T. MRM, 3013, 69:760. [5] Van Zijl & Yadav MRM 2011 65:927. [6] Vandeven F. et al. JMR (1988) 79: 221.

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