Magnetization Transfer of 5-FU in Model Systems

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Introduction:

Measurement of the concentration of drugs and drug metabolites is important for product development and dose determination. NMR is able to monitor delivery, clearance, and metabolism of 5-fluorouracil (5-FU) in vivo with 19-F spectroscopy. The sensitivity of 19-F spectroscopy is relatively low and limits temporal and spatial resolution. We present here a method to increase the sensitivity of detecting 5-FU in model systems by more than an order of magnitude by saturating the pyrimidine proton resonance of 5-FU and measuring changes in the water proton magnetization.

Methods:

Two samples of 70 mM 5-FU were made in D_2O; a neat solution of 5-FU, and a mixture of 5-FU and 15% bovine serum albumin (BSA). The BSA solution was cross-linked with glutaraldehyde. Proton NMR Spectra of the pyrimidine 6-H proton of 5-FU are shown in Fig. 1 for the solution (blue line) and the immobilized protein (red line) samples. A Magnetization transfer (MT) spectrum of the water proton magnetization was obtained from the cross-linked sample. Fig. 2 shows the full bandwidth MT spectrum and Fig. 3 shows the central region (±4kHz) of the MT spectrum in 100 Hz steps. All NMR experiments were done at 14T and 298 °C.

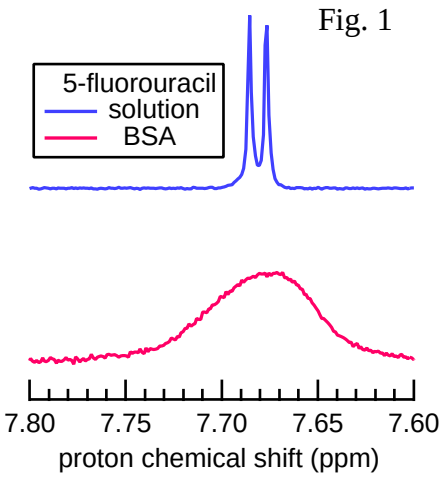

Fig. 1

Results and Discussion:

A scalar coupling (J=5.5 Hz) between the pyrimidine fluorine and detected proton resonance is observed in the spectrum of 5-FU in solution in Fig. 1. In immobilized BSA, the 5-FU proton T_2 shortens considerably due to exchange broadening (Fig. 1). This seemingly undesirable feature also provides a mechanism for the proton magnetization of 5-FU to couple to the proton magnetization of BSA. Fig. 2 shows a conventional MT spectrum of water in the cross-linked BSA sample with an additional sharp feature (drawn in red) near the direct water proton saturation at 0 Hz. Fig. 3 provides an expanded view of central region of the MT spectrum and highlights the additional RF saturation (again drawn in red). Also seen in Fig. 3 is direct water saturation at 0 Hz and an asymmetric line shape for the MT spectrum of BSA.

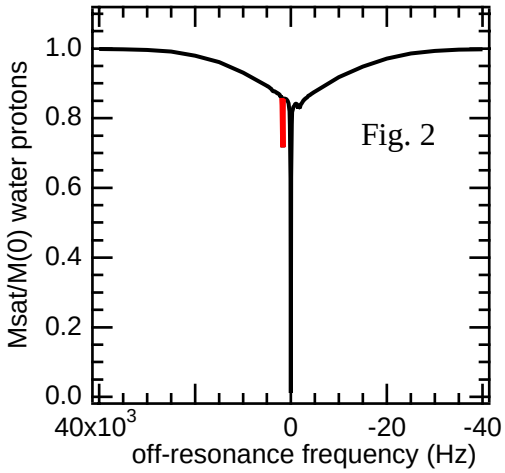

Fig. 2

Protein mediated, indirect magnetization transfer between 5-FU and water creates the sharp saturation feature observes in Figs. 2 and 3. The CW RF pulse saturates the 5-FU proton magnetization. Dipole-dipole interactions couple the saturated 5-FU magnetization to BSA, and finally to water.

The integrated intensity of the 5-FU signal in this sample is ~1.2% of the water signal (results not shown.) Fig. 3 shows that water proton magnetization is depleted by ~15% by indirect RF saturation through the 5-FU resonance. Therefore, this method is more than 10 times more sensitive than direct proton spectroscopy for detection of 5-FU in this particular model system.

In principle, the 1H-19F scalar coupling allows one to invert the 5-FU proton magnetization through the fluorine resonance. In practice, the small J coupling and short T_2 will make this method problematic. Techniques such as DEPT or INEPT require periods of 1/(2J) or 90 ms in order to achieve proton inversion. We are currently working on alternative methods to exploit the proton and fluorine magnetization in 5-FU.

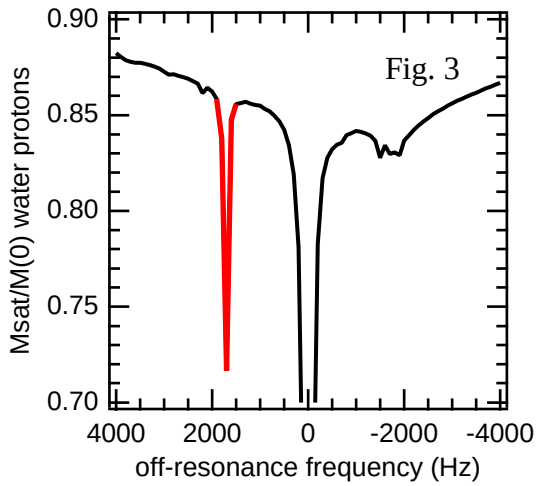

Fig. 3

Conclusion:

This work shows that the proton magnetization of 5-FU is magnetically coupled to BSA and hence to water. It is possible to indirectly detect 5-FU through the water proton resonance and achieve a factor of 10 gain in sensitivity. At this stage, we do not know if these mechanisms are also operative *in vivo*. Future work will be to show that these magnetic couplings exist *in vivo* and to develop methods to use the 19-F magnetization to provide either a saturation or inversion of the 5-FU proton magnetization.