Unexpected magnetisation transfer to aliphatic resonances in Z-spectroscopy in model systems and in vivo

P. L. Hubbard[1], J. Närväinen[2], R. A. Kauppinen[3], and G. A. Morris[1]

[1]School of Chemistry, University of Manchester, Manchester, United Kingdom, [2]A.I. Virtanen Institute, University of Kuopio, Finland, [3]School of Sport and Exercise Sciences, University of Birmingham, United Kingdom

Z-spectral asymmetry has been proposed as a quantitative source of pH-dependent contrast in MR images. Many labile protons exchange on an appropriate timescale to affect the bulk water signal, and it has been shown that the exchange rates of amide signals are sensitive to pH [1, 2]. At saturating RF amplitudes $\gamma B_2/2\pi$ above about 100 Hz, chemical exchange causes a decrease in water magnetisation when the amide protons are saturated, leading to a corresponding peak in the Z-spectral asymmetry (the difference between the Z-spectrum amplitude at corresponding points below and above the water Larmor frequency). It is shown here that in denatured bovine serum albumin (BSA) gels, at low saturating RF amplitudes a considerable intensity decrease is also evident in the aliphatic region of the Z-spectrum, apparently due to through-space interactions, potentially complicating the use of Z-spectral asymmetry. This is also seen in *in vivo* and is a potential source of error in pH imaging.

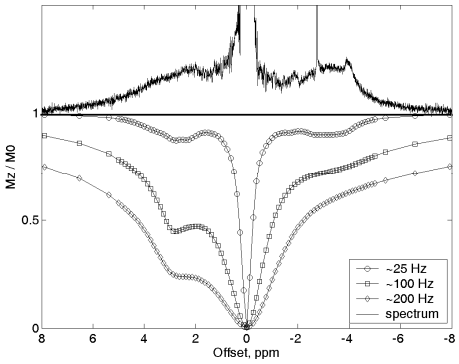

Fig. 1 Normalised Z-spectra of BSA at RF amplitudes of 25, 100 and 200 Hz, with (top) normal proton spectrum.

Methods BSA data were acquired at 9.4 T and 37°C on a Varian Inova spectrometer. Z-spectra were measured at saturation RF amplitudes of approximately 25, 100 and 200 Hz, using 20 s preirradiation followed by a small crusher gradient and a ~10° flip angle detection pulse (used in order to ensure approximately Lorentzian lineshapes irrespective of radiation damping). The probe was adjusted to exact electrical resonance, approximately 0.25 MHz from the point of minimum reflected power, as described earlier [3]. A total recycle delay of 35 s was used and the saturation frequency varied over ±100 kHz. Denatured BSA gels were prepared by heating 8 wt% BSA solutions in pH 5.5 phosphate buffer (20 mM, with 10% D_2O) at 60°C for 30 min. *In vivo* data were measured using a Magnex 4.7 T magnet interfaced to a Varian Inova console with a quadrature volume coil transmit, surface coil receive. Z-spectra were measured at saturation RF amplitudes of approximately 18 and 75 Hz, using 5 s preirradiation and a total recycle delay of 12 s. Data were collected from a total of 143 offsets varied over ±50 kHz and were read out along a 3 mm square column across the brain of a rat. Seven pixels (2 mm) from the cortical region were averaged.

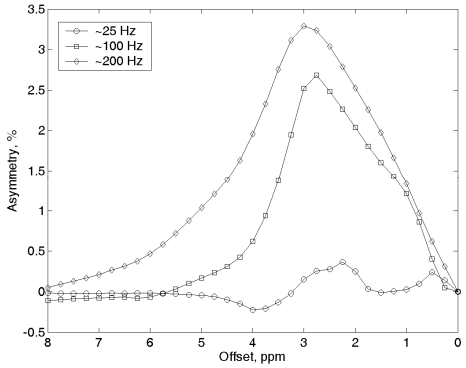

Fig. 2 Z-spectral asymmetry of BSA at RF amplitudes of 25, 100 and 200 Hz.

Results At low saturation RF amplitude moieties other than amides contribute to the features of a Z-spectrum. Figure 1 shows the Z-spectrum at a range of RF amplitudes, together with the normal ^{1}H spectrum (top). At 200 Hz, the Z-spectrum consists of a broad feature from direct water saturation, an amide exchange feature around 3 ppm above the water chemical shift, and a very broad MT background of ~20% saturation. At 25 Hz, the spectrum exhibits very little MT, the direct saturation feature is narrow, and there is clear saturation transfer from signals both above and below the water shift. The degree of saturation as a function of preirradiation frequency closely parallels the directly-observed spectrum (top), suggesting the presence either of a non-specific through-space interaction (i.e. a direct intermolecular nuclear Overhauser effect, NOE) or, more probably, of chemical exchange tightly coupled to efficient intramolecular spin diffusion. The Z-spectral asymmetry plots of Fig. 2 show clearly that at low preirradiation amplitudes it is not possible to identify, still less quantify, an amide exchange feature, the asymmetry plot reflecting a shifting balance between competing saturation at different chemical shifts. Figure 3 shows *in vivo* Z-spectra from rat brain at an RF amplitude (75 Hz) comparable to that currently used in pH imaging [1, 2, 4] and at lower amplitude (18 Hz). There is evidence of saturation transfer on either side of the water frequency, confirming the presence of this effect both in the BSA model and *in vivo*.

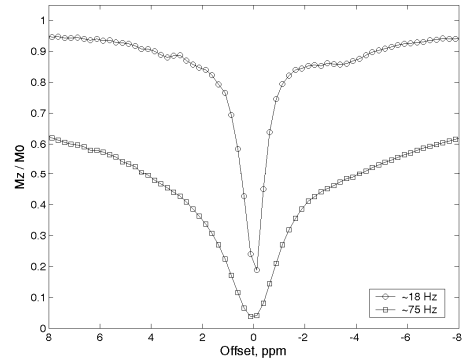

Fig. 3 Normalised *in vivo* rat brain Z-spectra at RF amplitudes of 18 Hz and 75 Hz.

Conclusion The observation of non-specific saturation transfer at low RF amplitudes in Z-spectra suggests that such effects may underlie the amide exchange features seen at higher amplitudes, potentially complicating the quantification of pH-dependent amide exchange.

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1. Zhou, J.Y., et al., Nat Med, 2003. **9**: 1085–1090.
2. Zhou, J.Y., et al. Magn Reson Med, 2003. **50**: 1120–1126.
3. Williamson, D.C., et al., J Magn Reson, 2006. **183**: 215–224.
4. Sun, P.Z., et al., J Magn Reson, 2005. **175**: 193–200.