MRS studies of spermine: factors affecting detectability

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Introduction

Magnetic resonance spectroscopy (MRS) offers a non-invasive means to characterise tissue based on its metabolic profile and may be used to provide diagnostic information. MR spectroscopic imaging has demonstrated a reduction of the spermine resonance in areas of prostate cancer[1,2]. To improve the accuracy of the MR measurement of spermine, this study investigates the effect of several chemical and physiological factors on its measurement. We report the effect of pH on the chemical shift (important for correct peak assignment), and the effect of protein and inorganic ions on T_2 relaxation and hence line width. We also report on the visibility of spermine in prostate cell lines and the efficiency of the methanol/chloroform (M/C) extraction method to extract spermine from cells.

Methods

Samples were prepared of 18mM spermine in (a) solutions of phosphate buffer with pH ranging from pH 7.0 to 8.0, (b) a solution mimicking prostatic fluid with inorganic ions (9mM $ZnCl_2$, 15mM $MgCl_2$, 18mM $CaCl_2$, 61mM KCl[1]) and 90mM citrate, (c) 100ml foetal calf serum (FCS) and (d) 1.4% by weight of albumin. All samples were made up to a total of 630µl with D_2O and 0.75% wt. TSP to act as a reference for chemical shift and for quantification.

PC-3 cells were grown in Dulbecco's Modified Eagle Media (D-MEM) with added pyruvate, L-glutamine and glucose (Gibco), LNCaP cells were grown in RPMI 1640 media. Cells were incubated at 37°C with 5% CO_2 for 3 days before harvesting approximately 30 – 70 million cells. M/C extraction of the cell cultures was performed by dissolving the cell pellet in 4ml each of methanol, chloroform and distilled water followed by vigorous stirring. The organic and inorganic layers were separated, freeze dried and then dissolved in 630µl D_2O (0.75% wt. TSP) for NMR analysis. A 1ml sample of 18mM spermine in distilled water was extracted in the same way using 1ml methanol and 1ml chloroform.

NMR experiments were conducted on a Bruker Avance 500MHz spectrometer (Bruker Instruments, Germany). ^{1}H NMR spectra were acquired using a 30 degree pulse-and-acquire sequence, with 128 to 512 acquisitions, at 298K. The T_2 measurements were carried out employing a standard CPMG pulse sequence, using 16 increments of echo times from 40ms to 1000ms.

Results

Effect of pH: The chemical shift of spermine decreases as the pH is increased (Fig. 1). At pH 7.2 the three peak areas of spermine are centred on 3.1ppm, 2.8ppm and, 1.8ppm, representing the fully protonated species. At this pH the 3.1ppm spermine peak is well resolved from choline (3.2) and creatine (3.0).

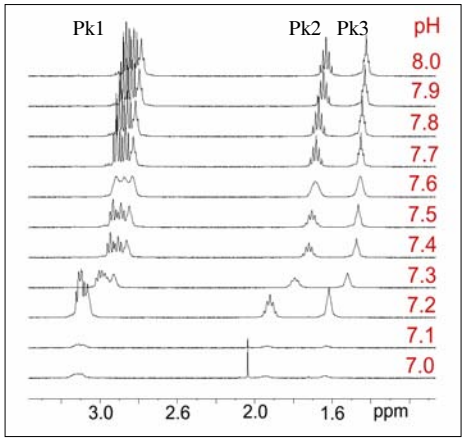

Figure 1: ^{1}HNMR spectra of spermine in D_2O/TSP of pH ranging from 7.0 to 8.0.

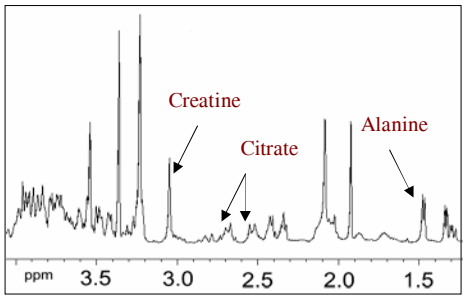

Fig 3. ^{1}H NMR spectrum of a M/C extract of LNCaP cell culture, pH 7.2.

Factors effecting T_2: The T_2 of 18mM spermine alone in D_2O was 775ms and decreases with the addition of FCS (540ms), of 1.4% wt. albumin (405ms) and with inorganic ions and citrate (166ms). A substantial modulation of the signal decay is observed at early echo times (Fig 2.) owing to evolution under the homonuclear ^{1}H-^{1}H scalar couplings. These are not refocused by the 180 degree pulses in the CPMG sequence and could give rise to substantial errors in the measurement of T_2 if not taken into account. Empirically the envelope of the decay becomes approximately exponential at echo times larger than 200ms and it is this decay which we have measured as T_2.

Effect of extraction methods: In Fig. 3 a ^{1}H NMR spectrum of a M/C extract of a LNCaP cell culture is shown. No spermine is detected in the LNCaP cell extracts of 3 x 10^7 cells and in PC-3 cell extracts of up to 1.3 x 10^8 cells. There was no significant loss in the extraction procedure, this was validated by the extraction of the sample of 18mM spermine; which showed 70% extracted in the methanol fraction, 20% in the chloroform fraction and 10% lost during the procedure.

Discussion and Conclusions

Local pH of the environment affects the ^{1}H NMR chemical shift of spermine; pH 7.2 avoids overlap with choline and creatine. The T_2 of spermine is reduced slightly by proteins and substantially by inorganic ions present in tissue and seminal fluid. Careful choice of echo time is required to minimise effects of homonuclear coupling. In M/C extraction approximately 70% of spermine is extracted in the methanol fraction and 20% in the chloroform fraction.

The intracellular spermine concentration is typically 10- to 50-fold lower than those of amino acids[3] and was below the detection limit in the PC-3 and LNCaP cell extracts.

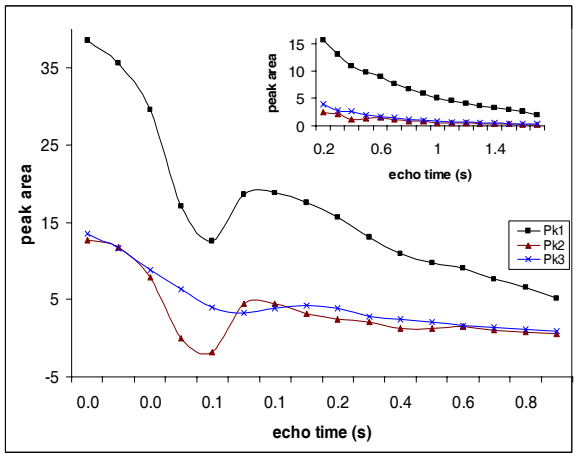

Fig 2. T_2 value for the three peaks of spermine with early echo times (below) and later echo times (top).

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[1] Marinette van der Graaf et al. MAGMA. 10 (2000) 153 – 159.
[2] Leo Ling Cheng et al. FEBS letters. 494 (2001) 112 – 116.
[3] Wieland Willker et al. NMR Biomed (1998) 11, 47 – 54.