Investigation of the CEST effect in prostate metabolites

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Purpose

The CEST effect has been used to image the concentration of several metabolites *in vivo*, for example, glucose, glutamate and glycogen. However, the CEST effect has not been investigated for the two predominant prostatic secretions: spermine (NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2) and citrate (HOOC-CH_2-C(OH)(COOH)-CH_2-COOH). These metabolites represent the secretory function of prostate tissues and so are of interest, as this normal function is reduced in tumour tissue. In this study, we investigate the CEST effect in spermine and citrate at physiological concentrations and pH levels.

Methods

CEST experiments were carried out on a Bruker 11.7T system using a 5mm BBO probe. For saturation, frequency-selective continuous-wave RF pulses were used with power 280Hz. A pulse length of 5 seconds was used as this achieved ~90% of maximum CEST effect at this power in tests, but kept scan time low. Z-spectra were acquired with saturation at 51 frequencies, using 0.2ppm offsets from -5.0ppm to +5.0ppm with respect to the water signal. *SpermineCEST (1) pH-dependence:* Published literature indicates that prostatic fluid has pH values in the range between 6.0 and 8.0 which are correlated to the citrate content (180mM-0mM, r = -0.64)[1]. Therefore, we measured the CEST effect in 10mM spermine solutions at 310K within these pH ranges. *(2) Spermine+CitrateCEST:* We measured the CEST effect in 10mM spermine solution with 100mM citrate at a range of pH values (4.0-8.0) at 310K. *(3) Chemical exchange rate-dependence:* To interrogate the chemical exchange rate *k* of spermine, we artificially lowered *k* by lowering the temperature of the sample (by 10K) and compared CEST at this temperature and at 310K. *CitrateCEST:* Finally, we measured the CEST effect in 100mM citric acid alone (pH 1.8).

Results

SpermineCEST (1) pH-dependence: A clear CEST effect was observed in spermine at pH values < 7.0 (Figure 1). The CEST asymmetry at pH 6.0 (the acidic limit of the prostate fluid pH range) is a maximum 41.8% at δ=+2.4ppm. This value dropped to 0.4% at pH 7.0 (similar to the noise level). *(2) Spermine + CitrateCEST:* CEST was reduced at all frequencies by the inclusion of citrate (Figure 2). At pH 6.0, the CEST asymmetry at δ=+2.4ppm in 10mM spermine + 100mM citrate solution was 6.1%. *(3) Chemical exchange rate-dependence:* By lowering *k* by reducing the solution temperature from 310K to 300K, the spermineCEST asymmetry *increased* from 41.0% to 54.2% (pH6.0, δ=+3.0ppm, Figure 3). This suggests that the spermine chemical exchange rate *k* moves into a more optimal CEST regime at 300K, when at 11.7T. *CitrateCEST:* No CEST asymmetry greater than 2% was observed from the 100mM citrate solution.

Figure 1: Z-spectra of 10mM spermine solutions at 310K at different pH levels

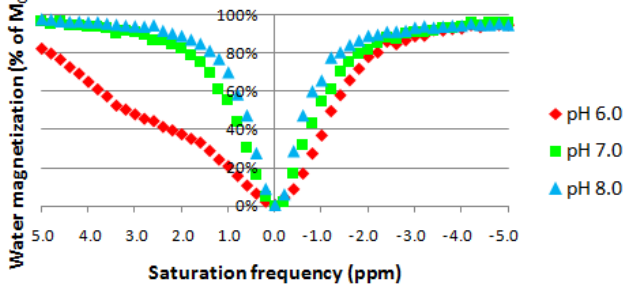


Figure 2: CEST asymmetry of 10mM spermine+100mM citrate solutions at 310K at different pH levels

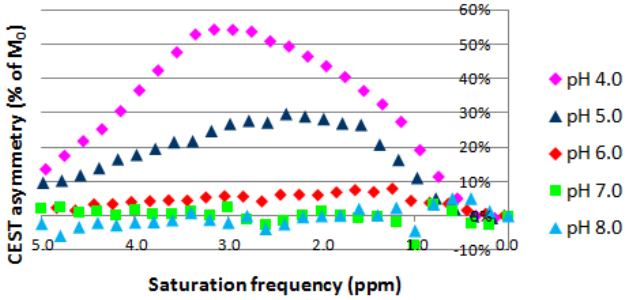
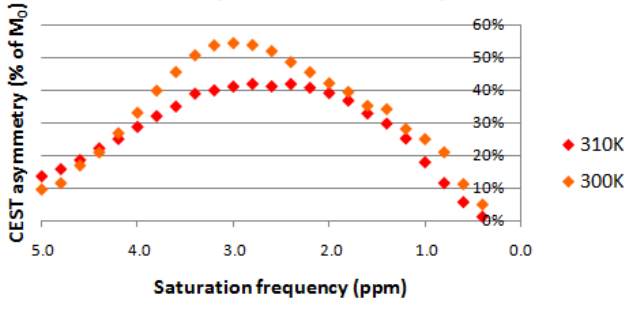


Figure 3: CEST asymmetry of 10mM spermine solutions at pH 6.0 at different temperatures



Discussion

We have shown that spermine demonstrates CEST under acidic pH conditions, as found in healthy prostate tissue.[2] The CEST effect occurs when the chemical exchange rate *k* of a spin species is slower than the chemical shift difference between the water protons and the spin species; this is called the slow exchange regime, $k \ll \Delta\nu$. CEST is dependent on *k* in this regime.[3] However, if the value of the chemical exchange rate, for any reason, increases and approaches Δν (intermediate exchange), the observed CEST is reduced. In the limit of fast exchange, $k \gg \Delta\nu$, CEST cannot be observed because the protons are exchanging too quickly with the water protons to be resolved. Spermine shows slow or intermediate chemical exchange under acidic conditions, but clearly the chemical exchange rate is dependent on pH levels, citrate levels and temperature in a non-trivial manner. The chemical origin of the CEST effect observed here in spermine is ambiguous, because spermine has two possible CEST spin species: NH *and* NH_2. The presence of two chemical exchange spin species means that there are two separate chemical exchange rates in spermine, not just one. This could account for the complex behaviours of spermineCEST at 11.7T; spermine displays an increased CEST asymmetry at acidic pH or lower temperature (Figures 1 and 3), but CEST asymmetry is reduced by the addition of extra citrate (Figures 2). Citrate had no observable CEST effect at 11.7T, so it is unknown why it would modify spermine's CEST activity. To investigate the chemical source of the spermineCEST effect further, we plan to measure the CEST effect in spermine's polyamine precursors, spermidine and putrescine, which retain the two NH_2 groups of spermine but which contain one and zero NH groups respectively. We will also investigate the CEST effect in solutions of varying amounts of both spermine and citrate.

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1. Kavanagh et al. J. Reprod. Fertil. **75**:35-41 (1985) 2 *Winnigham et al.* Nature **219**:139-143 (1968) 3 *van Zijl and Yadav* MRM **65**(4):927-948 (2011)