Determination of the pKa of a Hyperpolarized $H^{13}CO_3^-$ pH Probe

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Introduction: Tissue pH_e can be determined using ^{13}C MRS and hyperpolarized ^{13}C-labelled bicarbonate ($H^{13}CO_3^-$).[1] Using an assumed pK_a, the $H^{13}CO_3^-$ and $^{13}CO_2$ signal intensities allow pH determination using the Henderson-Hasselbalch equation. With a view to future clinical application we compared pH derived by DNP NMR with direct potentiometric measurement of H^+ activity using a glass electrode. Using $pK_a' = pH - \log([H^{13}CO_3]/[^{13}CO_2])$, we assigned the pH determined potentiometrically as the standard and determined whether the ^{13}C MRS derived pK_a diverges when there is alteration of ionic strength (I), pH, temperature and protein concentration within a clinically relevant range of values. Additionally, the pK_a in human blood was determined.

Method: Cs $H^{13}CO_3$ was prepared, polarized and dissolved using a method similar to that described previously.[1] Following dissolution, 2ml of 29mM $H^{13}CO_3^-$ was added to a 5ml phantom and the solution mixed. Phantoms consisted of a solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma), NaOH and NaCl to give a physiological I of 0.15. To investigate low ionic strength an alternative I of 0.05 was used. 12.5 Wilbur-Anderson units/ml of carbonic anhydrase (Sigma) was added to all phantoms. For experiments designed to simulate the range of pHs in tumors, the final pH was 6.7, 6.9 or 7.2 (measured immediately after addition of $H^{13}CO_3^-$). For all other phantom experiments the final pH was 7.1-7.2. For protein concentration experiments, 0g/dl, 1g/dl, 10g/dl or 25g/dl of bovine serum albumin (BSA, Sigma) were added. Samples were prepared at 37ºC and this temperature was maintained for the duration of each experiment. Additionally, samples maintained at 25ºC were investigated. A direct potentiometer (Radiometer) with a protein-compatible glass electrode (VWR) was calibrated with new buffer reagents using the manufacturer's temperature-pH scales. For human blood experiments a portable direct potentiometer (i-STAT, Abbott, NJ) was calibrated using the manufacturer's standards. After venepuncture, blood was heparinised and used immediately.

The NMR tube was placed in a 10mm broadband probe (Varian, Palo Alto, CA) tuned to 100.6MHz, in a vertical 9.4T, 8.9cm bore magnet (Oxford Instruments, UK) interfaced to a Varian INOVA spectrometer. Spectra of the hyperpolarized ^{13}C label using low flip-angle pulses (5º), 2µs pulse width, 1 sec repetition time, 1 transient per spectrum, 6 kHz spectral width collected into 2048 data points. 120 spectra were acquired and processed with 10Hz exponential line broadening.

Results:

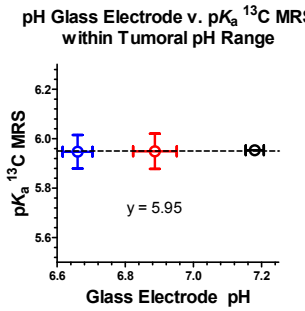

Figure 1: At each different pH value within the tumoral range (6.7, 6.9 or 7.2) the mean pK_a was 5.95 (n = 3 at each pH).

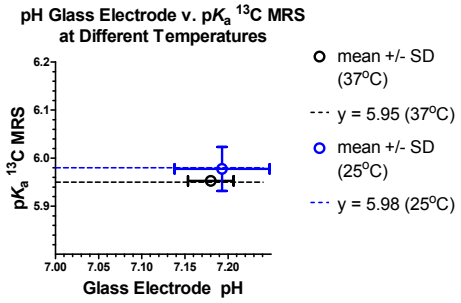

Figure 3: At 37°C the pK_a was 5.95 +/- 0.01. The pK_a was the same (5.98 +/- 0.05) at a temperature of 25°C, chosen to investigate the influence of hypothermia.

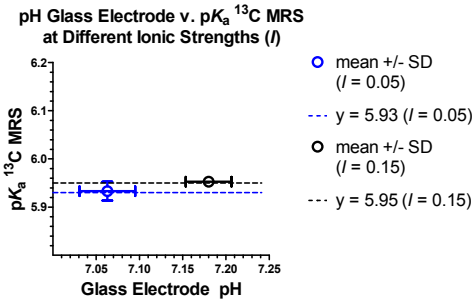

Figure 2: At an I equivalent to physiological serum or interstitium the ^{13}C MRS-derived pK_a was 5.95 +/- 0.01 (mean +/- S.D.; all phantom experiments n = 3). The pK_a was the same (5.93 +/- 0.02) when an I of 0.05 was chosen to investigate the influence of hyponatraemia (as may be seen in the cancer-related syndrome of inappropriate anti-diuretic hormone).

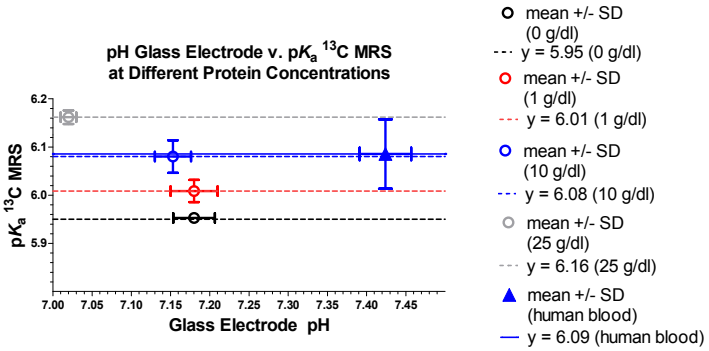

Figure 4: In protein-free samples (biologically similar to cerebral spinal fluid or urine) the pK_a was 5.95 +/- 0.01. In protein containing samples, the pK_a was slightly higher. For concentrations similar to interstitium and serous fluid (1g/dl), the pK_a was 6.01 +/- 0.02. For concentrations similar to serum or plasma (10g/dl), the pK_a was 6.08 +/- 0.06. The pK_a of human blood was almost identical, 6.09 +/- 0.07 (n = 5). For protein concentrations similar to pathological exudates (25g/dl), the pK_a was 6.16 +/- 0.01.

The pK_a in protein containing samples (calculated to change I <10%) was significantly higher than in protein-free samples: 0g/dl v. 1g/dl (2-tailed t-test, P = 0.02); 0g/dl v. 10g/dl (2-tailed t-test, P = 0.02); 0g/dl v. 25g/dl (2-tailed t-test, P< 0.0001). The pK_a in 25g/dl samples was significantly higher than in 1g/dl concentrations (2-tailed t-test, P = 0.0006).

Discussion: The negative logarithm of the apparent dissociation constant of carbonic acid ($H_2{}^{13}CO_3$) pK_a' is influenced by temperature, pH and ionic strength.[2,3] It is a composite constant including factors such as the activity and degree of dissociation of the $H^{13}CO_3^-$ ion.[2] It was not known whether a pK_a derived by ^{13}C MRS diverges from that derived by direct potentiometry within a clinically relevant range of values. The results demonstrated that within this range, the ^{13}C MRS-derived pK_a appears invariant to I, pH and temperature. We also showed that pK_a increases as protein concentration increases. However, given the small variation throughout an entire spectrum of clinically relevant protein concentrations this variation is unlikely to be clinically relevant for the purposes of imaging pH. If pH_e derived by ^{13}C MRS is weighted by capillary pH rather than tumor interstitial pH then it might be prudent to adopt a 'blood' or 'plasma' pK_a of 6.1 rather than an 'interstitial' pK_a of 6.0 for oncological imaging – this will ensure direct equivalence with pH determined potentiometrically.

[1]Gallagher FA et al Nature (2008) 453: 940-943. [2]Severinghaus J et al Appl Physiol (1956) 9:201-204. [3]Deane and Smith J Biol Chem (1957) 227: 101-106.