

Generation of hyperpolarized bicarbonate in large concentrations to image pH

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Purpose: In recent years, studies conducted on the hyperpolarization of nuclear spins have enabled enhancements of NMR signals by a factor of over 10^4 . This improvement has allowed high-resolution functional imaging of the lung as well as direct observation of pulmonary metabolism. Recently, it has allowed for the determination of *in-vivo* pH—an important parameter for the assessment of many fundamental pathologies. However, the application of directly polarized bicarbonate to pH imaging has been hindered by low levels of polarization and concentration. Here, we demonstrate that, by rapidly de-carboxylating the highly polarizable substrate pyruvate, we can produce large concentrations of two molecules that have been difficult to polarize directly – acetate and bicarbonate. These can then be used to simultaneously image pH and measure metabolism.

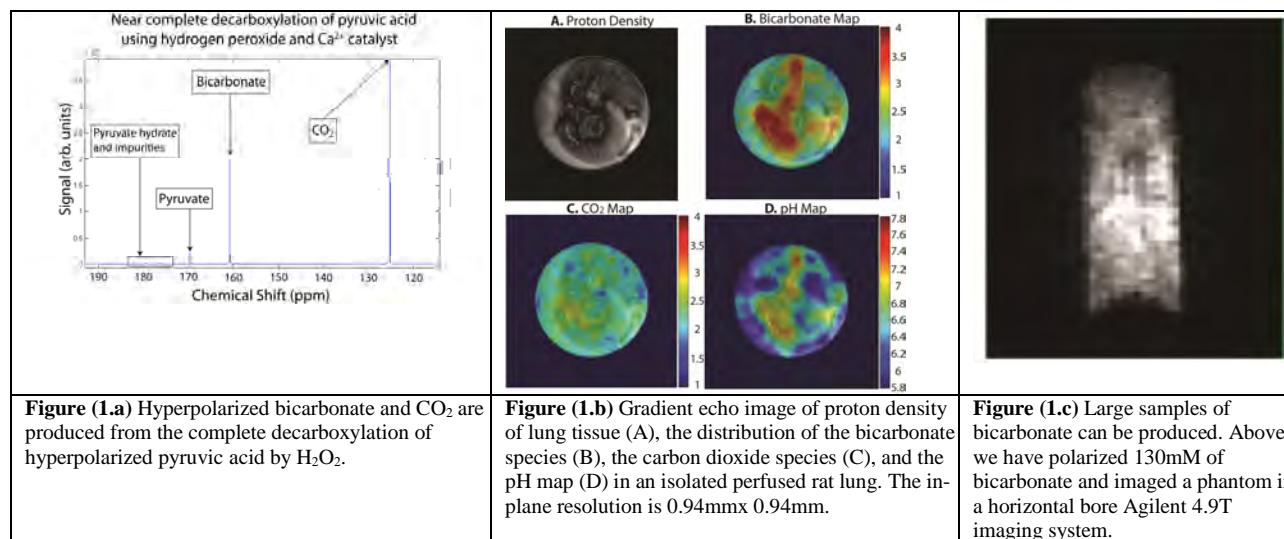
Methods: In general, the alpha keto carboxylic acids undergo rapid 2nd-order decomposition when catalyzed with H_2O_2 , as shown below¹:

$\text{R}-\text{CO}-\text{COOH} + \text{H}_2\text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{R}-\text{COOH}$. (eq. 1). The CO_2 undergoes a pH-dependent chemical equilibrium with bicarbonate, which is fast under typical aqueous conditions²: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$, $\text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$, $\text{HCO}_3^- \rightleftharpoons \text{CO}_3^{2-} + \text{H}^+$ (eq. 1). Under conditions of physiological pH, the HCO_3^- is the dominant form, and the products equilibrate in the ratio²: $\log_{10}([\text{CO}_2]/[\text{HCO}_3^-]) = \text{pH} - \text{pK}_a \sim 15.8$ (eq. 2). In order to rapidly capture the CO_2 and convert it to bicarbonate, carbonic anhydrase is added to the solution. Addition of concentrated base is required to keep the solution in the pH 7-8 range during the reaction. The reaction vessel was designed to exchange heat efficiently in order to reduce the peak temperature the reaction reaches.

Samples were hyperpolarized using a commercial DNP polarizer (Hypersense Oxford). OXO63 trityl radical was dissolved in 106-250mg of ¹³C-labeled substrate (1-¹³C Pyruvic acid, 1,2 ¹³C Sodium Pyruvate salt, and 1-¹³C alpha-keto isocitrate, Cambridge Isotopes) in a 1.7% by weight solution. The sample was subject to microwave irradiation at ~94.062 GHz at a temperature of 1.42K. The hyperpolarized sample was dissolved with 5.5ml of heated solvent under 10bar of pressure. The dissolution solvent contained 40mM of Trizma preset pH 7.6 crystals, 80mM of NaOH, and 50mM of NaCl (Sigma Aldrich). The dissolution buffer also contained 0.1g/l of disodium EDTA dehydrate to quench the paramagnetic OXO63 radical (Sigma Aldrich).

Imaging experiments were performed on two different systems. Perfused lung images were taken in a 9.4T vertical-bore Varian system and detected with a 5mm hetero-nuclear probe. Individual spectra were acquired with repetition times of 1s with tip angles less than 10° for ¹³C and 30° for proton spectra. Images of phantoms and *in-vivo* rat lung images were obtained in an Agilent 4.9T horizontal small animal imaging magnet. Tr's of ~6ms, and te's~2ms were used in conjunction with a frequency selective interleaved tof gradient echo imaging sequence.

The isolated rat lung was perfused at 10ml/min with a modified Krebs-Henseleit buffer. The buffer contained 119mM NaCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 4.7 mM KCl, 25mM NaHCO₃, 10mM glucose and 1% (w/v) bovine serum albumin. The perfusate was passed through and oxygenating column operating with 95:5 O₂/CO₂ under a constant flow of 1atm. The perfusate was kept at 37°C by encapsulating the flow tube in a constant temperature water jacket.



Results and Discussion: By varying the concentration of hydrogen peroxide, we can alter the decarboxylation to convert any fraction of the pyruvate to bicarbonate and acetate (see figure 1a). Co-production allows simultaneous monitoring of pH and pyruvate metabolism, as we have shown in an isolated rat lung model. The large concentrations of bicarbonate enabled sub-mm image resolution of pH (0.94mm x 0.94mm)(see figure 1b). In order to optimize the production of HP bicarbonate, several refinements are required. The pH of the reaction must be kept near constant during the reaction so as to not produce CO_2 in levels that exceed the solubility of the solution. pHs >8 slow the reaction rate, and pHs <7 lead to excess CO_2 bubbling out of solution. Additionally, the largely exothermic reaction must be conducted at low temperatures to ensure the CO_2 solubility does not decrease substantially and bubble out as well. This reaction can be performed on any alpha-keto carboxylic acid. For instance, the decarboxylation of alpha-keto isocitrate was observed to produce bicarbonate as well. Further substrates such as succinate and maloanate can be produced from alpha-keto glutarate and oxaloacetate, respectively.

Conclusion: Hyperpolarization of bicarbonate will make determination of pH *in vivo* practical. Bicarbonate is difficult to hyperpolarize directly, while its generation by decarboxylation of HP pyruvate is relatively straightforward. This technique results in concentrations up to 5 times higher than the largest HP bicarbonate sample produced to date, while eliminating the use of toxic alkaline salts. The increased concentration allows for sub-mm resolution of pH, a measurement that could be highly beneficial for treatment planning in cancer.

References: [1] KA. Nath, EO. Ngo, RP Hebbel, AJ Croatt, B Zhou, LM Nutter, Am J. Physiol 268(1Pt1):227-236, 1995. [2] FA. Gallagher, MI. Kettunen, KM. Brindle ,NMR in Biomedicine, Volume 24 Issue 8, pages 1006–1015, October 2011. [3] P.M. Mader, Journal of the American Chemical Society 80(11), pp 2634-2639 1958 [4] RK Ghosh, M Pourfathi, SJ Kadlec, RR Rizi, Magn. Reson. Med. (In press) November 2014