13C Succinate Catabolism in Breast Cancer Cells is Sensitive to Extracellular pH and Glucose and Glutamine Levels

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Introduction: Succinic acid metabolism by tumors is of interest because it can be hyperpolarized by the parahydrogen method (1). Transport of succinic acid across the cell membrane is mediated by dicarboxylic acid transporter proteins. Studies with Ehrlich Ascites Tumor Cells have demonstrated that succinate transport and oxidation is highly pH dependent (2). In addition, the flux of succinate into the TCA cycle will likely be strongly dependent on the availability of other substrates that can be used for cellular energy and biosynthesis. The goal of this work was to examine the effects of pH, glucose, and glutamine on succinate metabolism in a model breast cancer cell line. The results may be helpful for evaluating the suitability of hyperpolarized ^{13}C succinic acid for studying breast tumors.

Catabolism of succinic acid occurs in the TCA cycle and the malate shunt. It enters the TCA cycle at succinyl-CoA and is successively converted to succinate, fumarate, malate, oxaloacetate, citrate, isocitrate and alpha-ketoglutarate. To sustain the flux in the TCA cycle, two carbon units must be provided at the level of acetyl-CoA. With an active malate shunt, flux from the TCA cycle leaves as malate and is converted to pyruvate, which can be converted to lactate, alanine or acetyl-CoA. With conversion to acetyl-CoA, the TCA cycle can function normally to continue oxidation of succinate.

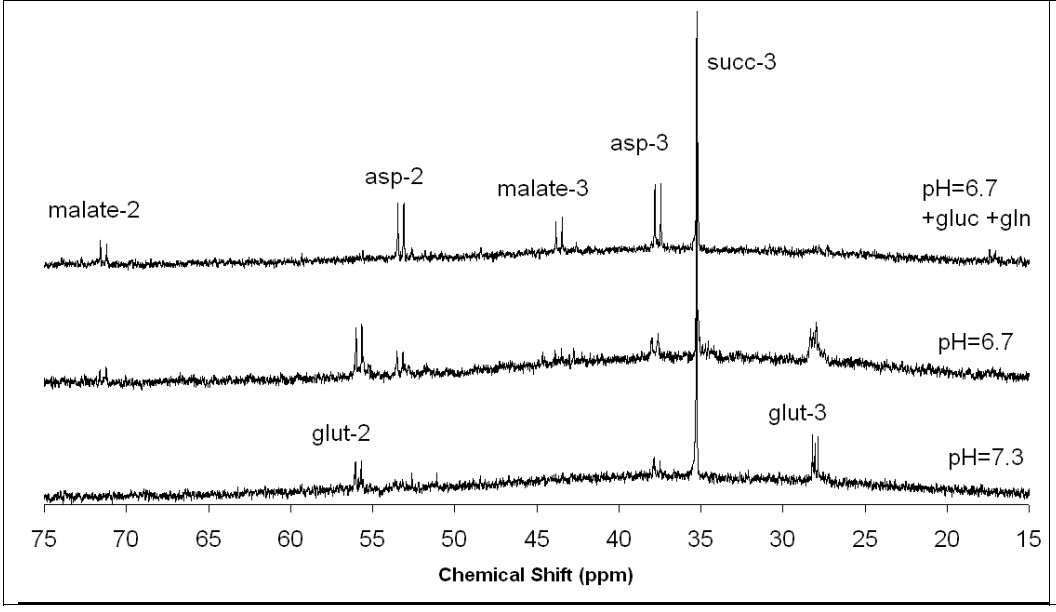
Methods: EMT6SF (mouse mammary tumor) cells were cultured in EMEM medium that was supplemented with 10% fetal bovine serum (FBS), 5 mM HEPES and penicillin/streptomycin. Cells were grown in a 5%CO_2/95% air incubator at 37 C, in T-flasks with a surface area of 225 cm^2. When they reached approximately 80% confluency (~1.5 x 10^7 cells), they were transferred to an oscillating platform in the incubator, which had a period of 6 seconds. The mild agitation ensured adequate oxygenation of the culture. For studies of succinate catabolism, [2,3-$^{13}C_2$]succinic acid was used (Cambridge Isotopes, Andover, MA, USA). The substrate was chosen because the T1 rates are relative rapid and would allow rapid evaluation of metabolite levels in cell extracts. For hyperpolarization studies, we anticipate using [1-^{13}C]fumaric acid to produce [1-^{13}C]succinic acid, which has a T1 of approximately 38 seconds in water. Succinate catabolism was studied in a buffer solution that contained 130 mM NaCl, 5 mM KCl, 1 mM MgCl, 1 mM $CaCl_2$ and 0.1% FBS. The initial succinate concentration was 3 mM. Cells were allowed to catabolize the succinate for 4 hours (done in duplicate flasks). Subsequently, they were washed twice with ice cold PBS and extracted with perchloric acid, according to the method of Bergemeyer (3). NMR spectroscopy was conducted with a 9.4 T Varian spectrometer and a 5 mm broad band probe. ^{13}C spectral parameters were: 60^o excitation, bi-level WALTZ16 ^1H decoupling, TR=2,600 ms, 65536 points, SW=25000 Hz, 40000 excitations, LB=1.5 Hz, 37 C.

Results and Discussion:

Spectra of extracts obtained from cells following catabolism of ^{13}C succinate are shown in the figure below. At pH 7.3, a moderate amount of label in glutamate C-2 and C-3 (reflecting label in alpha-ketoglutarate) was detected. The uncatabolized intracellular succinate served as a reference peak. Both glutamate resonances are doublets since they are coupled to each other. They were produced directly from carbons 2 and 3 of succinate by nearly one complete turn of the TCA cycle. An additional resonances within the glutamate C-3 resonance was observed may have been C-3 of pyruvate. A small amount of label was also detected in asparate C-2 and C-3. No label was detected in lactate, since unlike glucose, succinate is a relatively hydrogen poor substrate and only produces pyruvate through the malate shunt. It does not produce excess NADH. At pH 6.7, increased labeling was observed in C-2 and C-3 of glutamate and C-2 and C-3 of aspartate. A low level of label was also observed in both C-2 and C-3 of malate. Also, a small amount of label was observed in C-4 glutamate (34.3 ppm), indicating full malate shunt activity. Addition of 5 mM glucose to a culture at pH 6.7 reduced the level of labeling in C-2 and C-3 of glutamate (not shown). Addition of 5 mM glucose and 0.6 mM glutamine (values typically found in blood) completely eliminated labeling in glutamate and enhanced labeling in both aspartate and malate. Also, a small amount of label was detected in C-3 alanine at 17 ppm.

Conclusions:

The results demonstrate that both pH and glucose and glutamine levels are important in succinate catabolism. Low pH enhances succinate transport and increases 13C in the TCA cycle. Glucose and glutamine reduce labeling in glutamate but in combination increase labeling in malate and aspartate. Thus, hyperpolarized succinate may have some applications in identifying tumors with low pH or possibly high anaplerotic rates, where extracellular glutamine levels will likely be very low.



References:

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(3) Bergemeyer Methods of Enzymatic Analysis (184).