

Supporting Information

Hyperpolarised Organic Phosphates as NMR Reporters of Compartmental pH

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Experimental details

1. Reference spectra

Pyruvate, glyceronephosphate, 6-phosphogluconate and 3-phosphoglycerate salts were purchased from Sigma Aldrich (St Louis, Missouri, USA) and dissolved to concentrations of 20 mM in 100 mM potassium phosphate buffer (in 95% H₂O/5% D₂O) of pH 6.5 to yield final concentrations of alkali ions resembling the intracellular milieu (approximately 300 mM). One-dimensional ¹³C spectra were acquired on a Bruker (Fällanden, Switzerland) 800 MHz Avance II spectrometer equipped with a TCI Z-gradient cryoprobe and an 18.7 T magnet (Oxford Magnet Technology, Oxford, U.K.) by sampling 16384 complex data points during an acquisition time of 330 ms. The pH was varied by titration with 1 M NaOH and determined after each NMR measurement.

2. Cell culture

Precultures of the *S. cerevisiae* yeast strain BY 4743 were inoculated from a YPD plate in 20 ml YPD medium (2% w/v glucose, in 100 ml Erlenmeyer flasks) and were grown over night at 30°C (200 rpm shaker speed). Main cultures were inoculated from the precultures to OD⁶⁰⁰=0.1 in YPD medium (100 ml culture in 500 ml Erlenmeyer flasks) and were grown to exponential phase (OD⁶⁰⁰≈ 1) at 30°C and 200 rpm shaker speed. From these cell cultures, 50 ml were harvested by centrifugation for 5 min at 5000 g in Falcon™ tubes; the supernatants were discarded and the cell pellets were resuspended in 2.5 ml of 50 mM MES buffer of pH 5.6 or acetate buffer (10, 23 or 35 mM) of pH 4.5. Finally, the resuspended cells were transferred to a 10 mm NMR tube in which they were thermally equilibrated at 30°C (5 min) prior to the injection of hyperpolarized [U-²H, U-¹³C]glucose. Experiments were conducted with 3.5 mM hyperpolarized [U-²H, U-¹³C]glucose as substrate.

3. Hyperpolarization and in-cell NMR

Substrate samples for nuclear spin polarization enhancement were prepared by dissolving 16 mg of [U-²H, U-¹³C]glucose (Sigma Aldrich) in 17 mg of an aqueous polarization medium containing 27 mM trityl radical OX063 (Oxford Instruments, UK) and 0.9 mM trimeric Gd chelate of 1,3,5-tris-(N-(DO3A-acetamido)-N-methyl-4-amino-2-methyl- phenyl)-[1,3,5]tria-zinane-2,4,6-trione (GE Healthcare, Amersham, UK). The samples were vitrified by flash freezing in liquid

helium inside a 3.35 T polarizer. Nuclear spin polarization enhancement was conducted by microwave irradiation at 93.89 GHz and 100 mW for 50 min at 1.2 K. Hyperpolarized substrate samples were dissolved in 4.5 ml Milli-Q water.¹ NMR data acquisition was started prior to substrate injection to cell suspensions residing inside a 600 MHz NMR spectrometer. Of the hyperpolarized [U-²H, U-¹³C]glucose solutions, 600 μ l (18 mM) were rapidly injected by hand into 2.5 ml cell suspension.^{2,3}

Notably, these experiments are run without the presence of deuterated solvent or reference standard compounds. As a result, we suggest using internal relative referencing to the intracellular pyruvate signal for pH determinations.⁴ Equivalently, the pyruvate signal with a ¹³C₂ chemical shift of 207.8 ppm relative to a TMS standard can be used to transfer the relative to an absolute scale.

4. Data acquisition and processing

In-cell NMR spectra were recorded on a Bruker DRX 600 spectrometer equipped with a 10 mm BBO room temperature probe head. 512 one-dimensional ¹³C NMR spectra were recorded as a pseudo-2D with 6° flip angle pulses and by summing two transients per spectrum. Spectra were recorded every 500 ms. 16384 data points were sampled in the time domain. The spectra were processed with an exponential line broadening of 20 Hz in Topspin 3.0. For an improved signal to noise ratio, sum spectra of 40 one-dimensional spectra (0-20 seconds) were extracted as shown in main text figure 5. The 3-phosphoglycerate signal was weaker than the pyruvate and glyceronephosphate signals. Due to the detrimental effect of acetic acid on cytosolic pH and cellular metabolism, chemical shift differences were therefore only extracted for pyruvate and glyceronephosphate signals and treated with main text equation 1 to yield the results of Table S1.

Table S1. Chemical shift differences between glyceronephosphate and pyruvate in the cytosol of living yeast at 30°C at varying concentrations of acetic acid.

[acetic acid]/mM	$\Delta\delta_{\text{obs}}/\text{ppm}$	pH _{cyt} ^a
0.00	5.99	6.40
6.40	5.53	5.92
14.70	5.36	5.77
22.40	5.15	5.58

^aCytosolic pH values extracted with main text equation (1).

References

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