Electronic Supplementary Material (ESI) for Molecular BioSystems

Real-time visualization of central carbon metabolism in living yeast by DNP-NMR

Supplemental information

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Experimental details

Cell culture

Saccharomyces cerevisiae strain BY4743 *(MATa/MATα; his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0)* is a parent strain of the international *S. cerevisiae* gene disruption project.[1] Cells were grown as batch cultures in 100 mL YPD medium containing 2%(w/v) glucose by inoculation to OD^{600}=0.1 with starter cultures grown over night. Cultures were grown in Erlenmeyer flasks to OD^{600}=3 at 30°C and subsequently adapted to YPD medium containing 0.4% (w/v) glucose for 90 minutes. Cells were harvested by centrifugation and were resuspended to a total volume of 2.4 ml in fresh YP medium prior to injections of DNP enhanced carbohydrate. *In vivo* DNP NMR experiments worked equally well upon resuspension of yeast cells in supernatant growth medium. Cells were not aerated at any stage.

DNP preparation

Carbohydrates are expected to be well-suited substrates for DNP due to their high tendency to form glasses.[2,3] Formation of glasses is preeminent in permitting efficient microwave-driven polarization transfer from an electron spin to an analyte of interest in a homogenous sample. Optimized DNP preparations were achieved by dissolving 90 μmol of [U-^{2}H, U-^{13}C]glucose or [2-^{13}C]fructose in 19 mg of aqueous polarization medium containing 27 mM trityl radical OX063 and 0.9 mM trimeric Gd complex. Samples were frozen in liquid helium and polarization transfer was performed for one hour at 1.2 K by microwave irradiation at 93.89 GHz with 100 mW in a magnetic field of 3.35 T to yield

solid state polarizations on the order of 30% for [U-^{2}H, U-^{13}C]glucose and 33% for [2-^{13}C]fructose. DNP enhanced substrates were dissolved in 4.5 ml water containing 100 mg/L EDTA. 600 µl of the dissolved substrate was forcefully injected into 2.4 ml *S. cerevisiae* cell suspension residing in a 10 mm sample tube inside a shimmed NMR magnet at 30°C. Final concentrations of [2-^{13}C]fructose or [U-^{2}H, U-^{13}C]glucose applied to cell suspensions were thus 4 mM. In the dissolved sample, [2-^{13}C]fructose yielded a time constant for spin polarization loss of T_1{14.1 T}≈27 s, while [U-^{2}H, U-^{13}C]glucose yielded T_1{14.1 T}≈12 s for $^{13}C_{1\text{-}5}$ and 10 s for $^{13}C_6$. The use of [2-^{13}C]fructose thus leads to increased sensitivity of the *in vivo* assay due to the long T_1 time of the quaternary carbon and due to absence of ^{13}C-^{13}C spin couplings.

***In vivo* spectroscopy**

NMR spectra were recorded on a Bruker DRX 600 spectrometer equipped with a 10 mm room temperature probehead. ^{13}C NMR spectra were recorded as an array of 512 one-dimensional spectra with low flip angle (6°). Acquisition of spectra was initiated prior to DNP dissolution and sample injection. 16384 data points were sampled during 170 ms in the time domain. ^{13}C NMR spectra of [U-^{2}H, U-^{13}C]glucose metabolism were recorded with a temporal resolution of 500 ms by summing two transients. Spectra were processed with an exponential line broadening of 20 Hz except for the 90-110 ppm region in **Fig. 3**, which was processed with a negative line broadening of 1 Hz and a maximum of the Gaussian function at 0.3 in order to resolve the fructose-1,6BP-C_2 and DHAP-C_2 hydrate signals in presence of the substrate signal. Signals were integrated with Bruker Topspin 2.1. Despite of vast signal enhancements, improved sensitivity for the detection of cellular reaction chemistry remains an ambition. Use of high field DNP polarizers, fast substrate injection systems and detection with cryogenically cooled NMR probes could allow improvements by an order of magnitude and reduce the required cell densitiy for *in vivo* NMR accordingly.

References

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