NMR measurement: Sample preparation and analysis

For studies on extracellular media, 5TGM1, OPM2 and U266 cells were incubated in the presence of 0.005-0.01 M 1-^{13}C or 2-^{13}C Acetate in standard growth at a concentration of 10^6 cells/mL (-/+0.35). Supernatants were collected at the indicated time-points by centrifugation and snap-frozen in liquid nitrogen. 1 mL cell growth media was frozen with dry ice then lyophilized by Speed Vac Concentrator (Savant®). The lyophilized samples were saved at – 80 °C until use. Samples were re-suspended into 0.56 mL D_2O with 1 mM t-butanol as an internal standard. The sample pH was adjusted to 7.0 and then loaded into 5 mm tubes for NMR analysis. Cell extraction was performed as previously described (1) (2). Briefly, cells were extracted with 0.5 mL -20 °C 50% methanol/water incubation for 20 min on dry ice, sonicated for 1-2 minutes with 30 off/on cycles (50% duty cycle) in an ice bath. 0.5 mL chloroform was added and centrifuged at 4 °C, 13000-15000 rpm for 10 min. Both aqueous and chloroform layers were collected and dried for NMR analysis. The metabolites from aqueous layer were re-suspended into 0.3 mL D_2O with 1 mM t-butanol internal standard and charged into a susceptibility-matched Shigemi tube. The metabolites from chloroform (membrane extraction) were re-suspended in 0.6 mL deuterated chloroform.

The NMR measurements were carried out at 25 °C using a DD-II 11.75 tesla spectrometer (Agilent/Varian) equipped with a reverse-detection probe. Two measurements were carried out on all samples. The first was a 16-transient CPMG (Car-Purcell-Meiboom-Gill) with water pre-saturation and ^{13}C decoupling (where proton doublets bounded to ^{13}C are collapsed to singlets at a chemical shift identical to protons bound to ^{12}C) under quantitative equilibrium conditions. The CPMG method was chosen to rid signals from macromolecules through appropriate choice of a T_2 filter. Collection conditions include a spectral window of 6983 Hz, ^{1}H 90° pulse width of 6.9-8.4 µs (pending salt), 1 s acquisition time, 14K complex data points., 18 s pre acqusition delay and 4

dummy scan). Spectra were commonly processed with an exponential apodization function corresponding to 1 Hz line-broadening factor and zero-filling to 64K. Substrate and metabolite concentrations were determined from the CPMG data through prior calibration relative to 1 mM t-butanol established by equilibrium pulse and collect conditions. The second NMR measurement is a first-increment gHSQC experiment to effectively measure only the ^{13}C-enrichment of substrate and metabolites. This acquisition is collected for 128 transient under quantitative equilibrium conditions similar to the CPMG, with ^{13}C a 90° pulse width of 12 μs. The FID values were multiplied by a gaussian adapodization function with a 0.1 s time constant. The relative amplitude of substrates or metabolites was calculated with Agilent CRAFT (Complete Reduction to Amplitude-Frequency Table) software installed in VnmrJ4.2.

For the ^{1}H-^{13}C gHSQC 2D experiment measurement on lipid layer from cell extractions, the acquisition is collected for 16 transient in t_2 with 160 increments in the second dimension (t_1) and 4 dummy scans. The ^{1}H 90°pulse width is 6.5 μs; the ^{13}C 90° pulse width is 12μs. The spectral width is 8012 hz for ^{1}H and 31250 hz for^{13}C. The FID was manipulated via an apodization function consisting of a negative exponential and 0.05 hz Gaussian function. The spectra are processed with VnmrJ 4.2A software.

REFERENCES

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